STIC-Biotech/ChemLib 143154 ME

From: Portner, Ginny
Sent: Tuesday, January 25, 2005 10:07 AM
To: STIC-Biotech/ChemLib
Subject: 09/904,994

Please search SEQ ID NOs 1-3, probes, primers, oligomers and interference search both NA and protein sequences. thanks

Ginny Portner
Remsen Building
Art Unit 1645
Room E03, B02
(571) 272-0862

****
STAFF USE ONLY

Searcher:_____
Searcher Phone: 2-
Date Searcher Picked up:____
Date Completed:_____
Searcher Prep/Rev. Time:___
Online Time:_____

****
Type of Search
NA Sequence: #_____
AA Sequence :#_____
Structure: #_____
Bibliographic:_____
Litigation:_____
Patent Family:_____
Other:_____

****
Vendors and cost where applicable
STN:_____
DIALOG:_____
QUESTEL/ORBIT:_____
LEXIS/NEXIS:_____
SEQUENCE SYSTEM:_____
WWW/Internet:_____
Other(Specify):_____

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Thursday, June 09, 2005

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L1	felis and urease	137
☐	L2	(felis and urease).ti,ab,clm.	29
		DB=USPT; PLUR=YES; OP=AND	
☐	L3	US-6248330-B1.did.	1
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L4	(felis).ti.	19
		DB=USPT; PLUR=YES; OP=AND	
☐	L5	(cs1 or cs-1 or kukka or ds4 or ds-4 or 2301 or 49179) and felis	22
☐	L6	(cs1 or cs-1 or kukka or ds4 or ds-4 or 2301 or atcc49179 or atcc-49179 or 49179) and felis	22

END OF SEARCH HISTORY

. 6849419. 23 Jul 02; 01 Feb 05. Monoclonal antibody hybridoma immunoassay method and diagnosis kit. Wakasugi; Masahiko, et al. 435/7.32; 435/14 435/27 435/28 435/29 435/34 435/4 530/388.1 530/388.2. C12N015/02.

☐ 2. 6800744. 30 Jun 98; 05 Oct 04. Nucleic acid and amino acid sequences relating to Streptococcus pneumoniae for diagnostics and therapeutics. Doucette-Stamm; Lynn A., et al. 536/23.1; 435/320.1 435/325 435/419 435/6 536/23.4 536/24.1 536/24.32. C12Q001/68 C12N001/14 C12N015/00 C12N005/00 C12N005/04 C07H021/02 C07H021/04.

☐ 3. 6750256. 29 Oct 99; 15 Jun 04. Use of aromatic aldehydes as insecticides. Crandall, Jr.; Bradford G., et al. 514/701; 424/405 424/406 514/693. A01N035/00.

☐ 4. 6713279. 04 Feb 00; 30 Mar 04. Non-stochastic generation of genetic vaccines and enzymes. Short; Jay M.. 435/69.1; 435/320.1 435/334 435/6. C12P021/06 C12Q001/68 C12N005/06 C12N015/00.

☐ 5. 6663873. 09 Aug 96; 16 Dec 03. Antigenic preparation for treatment or prevention of helicobacter infection. Doidge; Christopher Vincent, et al. 424/234.1; 424/130.1 424/137.1 424/141.1 424/150.1 424/184.1 424/193.1 424/278.1 424/283.1 424/434 424/93.4 514/12 514/2 514/54. A61K039/00 A61K039/02 A61K045/00 A61K039/385.

☐ 6. 6617156. 13 Aug 98; 09 Sep 03. Nucleic acid and amino acid sequences relating to Enterococcus faecalis for diagnostics and therapeutics. Doucette-Stamm; Lynn A., et al. 435/320.1; 435/252.3 435/6 435/69.1 536/23.7 536/24.32. C12N015/31 C12N015/63 C12N001/13 C12Q001/68.

☐ 7. 6576757. 28 Nov 00; 10 Jun 03. Polynucleotides encoding flavivirus and alphavirus multivalent antigenic polypeptides. Punnonen; Juha, et al. 536/23.72; 424/184.1 424/204.1 424/218.1 424/228.1 536/23.1. C07H021/04 A61K039/12 A01N043/04.

☐ 8. 6569435. 28 Nov 00; 27 May 03. Flavivirus and alphavirus recombinant antigen libraries. Punnonen; Juha, et al. 424/202.1; 424/204.1 424/234.1 424/236.1 424/274.1 435/320.1 435/6 514/44. A61K039/12 A61K039/295 A01N043/04.

☐ 9. 6541011. 10 Feb 99; 01 Apr 03. Antigen library immunization. Punnonen; Juha, et al. 424/204.1; 424/218.1 530/300 530/350. A61K039/12 C07K001/00.

☐ 10. 6432948. 08 Nov 99; 13 Aug 02. USE OF 7-(2-OXA-5,8-DIAZABICYLCO[4.3.0]NON-8-YL)-QUINOLONE CARBOXYLIC ACID AND NAPHTHYRIDON CARBOXYLIC ACID DERIVATIVES FOR THE TREATMENT OF HELIOBACTER PYLORI INFECTIONS AND ASSOCIATED GASTRODUODENAL DISEASES. Matzke; Michael, et al. 514/229.2; 514/230.2 514/230.5 544/105 544/66 544/73. A61K031/536 C07D265/28.

☐ 11. 6406703. 05 Jul 00; 18 Jun 02. Treatment of H. pylori associated gastroduodenal disease. Doidge; Christopher Vincent, et al. 424/234.1; 424/194.1 424/203.1 424/450 424/457 424/460 424/461 424/500 424/501 424/502 530/350. A61K039/02 A61K039/16 A61K009/64 A61K039/85 C07K001/00.

☐ 12. 6288081. 06 Aug 99; 11 Sep 01. Use of 7-(1-aminomethyl-2-oxa-7-aza-bicyclo[3.3.0]oct-7-yl)-quinolone carboxylic acid and naphthyridone carboxylic acid derivatives for treating Helicobacter pylori infections and the gastroduodenal diseases associated therewith. Petersen; Uwe, et al. 514/312; 514/229.2 514/230.2 514/291 514/292 514/293 514/300 544/101 544/66 546/123 546/156 546/80 546/81 546/83 546/84 548/453. A61K031/470 A61K031/437 C07D215/56 C07D471/04 A61D001/04.

☐ 13. 6261769. 31 Mar 98; 17 Jul 01. Intergenic spacer target sequence for detecting and distinguishing Chlamydial species or strains. Everett; Karin D. E., et al. 435/6; 435/91.2 536/24.32 536/24.33. C12Q001/68 C12P019/34 C07H021/04.

☐ 14. 6258359. 06 Jun 95; 10 Jul 01. Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides. Labigne; Agnes, et al. 424/141.1; 424/150.1 424/163.1 424/164.1 530/350 530/388.1 530/388.2 530/388.4. A61K039/395 A61K039/40 C07K001/00 C07K016/00.

☐ 15. 6248330. 02 May 95; 19 Jun 01. Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides. Labigne; Agnes, et al. 424/192.1; 424/184.1 424/234.1 435/6 435/69.1. A61K039/00.

☐ 16. 6245516. 07 Dec 99; 12 Jun 01. DNA probes for campylobacter, arcobacter and helicobacter. Al Rashid; Shahnaz Tahihra, et al. 435/6; 435/91.2 536/23.1 536/24.3. C12Q001/68 C12P019/34 C07H021/02 C07H021/06 C12N015/00.

☐ 17. 6133260. 14 Jun 99; 17 Oct 00. Use of 7-(2-oxa-5,8-diazabicyclo[4.3.0]non-8-yl)-quinolone carboxylic acid and naphthyridon carboxylic acid derivatives for the treatment of Helicobacter pylori infections and associated gastroduodenal diseases. Matzke; Michael, et al. 514/230.5; 544/105. C07D265/28 A61K031/5365.

☐ 18. 6129923. 29 Oct 98; 10 Oct 00. Treatment of H. pylori associated gastroduodenal disease. Doidge; Christopher Vincent, et al. 424/234.1; 424/194.1 536/23.1. A61K039/02.

☐ 19. 6060241. 03 Apr 97; 09 May 00. Compositions and methods relating to drug discovery and detection and treatment of gastrointestinal diseases. Corthesy-Theulaz; Irene. 435/6; 536/23.2. C12Q001/70.

☐ 20. 5871749. 18 Aug 95; 16 Feb 99. Therapeutic treatment of H. pylori associated gastroduodenal disease. Doidge; Christopher Vincent, et al. 424/234.1; 424/194.1 435/7.21. A61K039/02.

☐ 21. 5843460. 06 Jun 95; 01 Dec 98. Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides. Labigne; Agnes, et al. 424/234.1; 435/6 435/7.32 435/7.9 514/234.5 514/41. A61K039/02.

☐ 22. 5610060. 24 Jun 94; 11 Mar 97. Isolated Helicobacter hepaticus. Ward; Jerrold M., et al. 435/252.1; 435/243. C12N001/20.

Generate Collection Print

Terms	Documents
(cs1 or cs-1 or kukka or ds4 or ds-4 or 2301 or atcc49179 or atcc-49179 or 49179) and felis	22

Prev Page Next Page Go to Doc#

ATCC®
The Global Bioresource Center™

Your Discoveries Begin with Us.™

Search: --- Choose Op

Search

The search engine found 2 entries for *helicobacter & felis*.

Entries 1 to 2 are currently being displayed. Continue Search.

View Selections | View All | Clear Selections

Bacteria

ATCC® Number	Description	Designation	View
49179	*Helicobacter felis* Paster et al. deposited as Cat spiral 1	CS1	☐
51211	*Helicobacter felis* Paster et al.	CS6	☐

View Selections | View All | Clear Selections

You may continue your word search in Bacteria selections by typing in your search criteria below or returning to the Bacteria menu. To search another product line, choose one from the dropdown box at the top. For complex searches using boolean operators, the following characters must be used: & (for AND), | (for OR), ^ (for AND NOT). An asterisk (*) is used as the wildcard. For more information please review the Search Help.

helicobacter & felis | Word Search | Clear Search

Home Page Archive

Table 1b:

amino acid and nucleic acid homology between the *H. felis* ureY and various ureB subunits.		
Reference molecule : H. *felis* ureY CS1	a.a.	n.a.
H. felis ureB	73 %	71 %
H. pylori ureB	73 %	70 %
H. heilmannii ureB	74 %	71 %
H. felis strain Kukka ureY	99 %	95 %
H. felis strain Ds4 ureY	98 %	94 %
H. felis strain 2301 ureY	99 %	95 %

Table 1c:

nucleic acid homology between *H. felis* ureXY and various ureAB genes.	
Reference molecule: *H. felis* ureXY CS1	n.a.
H. felis ureAB	67 %
H. pylori ureAB	67 %
H. heilmannii ureAB	68 %
H. felis strain Kukka ureXY	94 %
H. felis strain Ds4 ureXY	94 %
H. felis strain 2301 ureXY	94 %

49179

[0006] One embodiment of the invention thus relates to nucleic acid sequences encoding the novel urease X and Y subunits.

[0007] First of all, this embodiment of the invention relates to nucleic acid sequences encoding two subunits of a urease complex such as expressed by *Helicobacter felis,* that have at least 85 % homology with SEQ ID NO: 1, or parts thereof with a length of at least 40, preferably 45, more preferably 50 nucleotides encoding at least an immunogenic fragment of one of the subunits. Still even longer fragments, with a length of at least 55, 60 or 70 nucleic acids are in that order even more preferred.

[0008] A preferred form of this embodiment relates to nucleic acid sequences that encode the urease X subunit polypeptide or the urease Y subunit polypeptide and that have at least 85 % homology with SEQ ID NO: 1, or parts thereof with a length of at least 40, preferably 45, more preferably 50 nucleotides encoding at least an immunogenic fragment of the urease X subunit polypeptide or the urease Y subunit polypeptide. Merely as an example: the nucleic acid sequence encoding the urease X subunit of *Helicobacter felis* strain CS1 starts at position 206/207/208 (GTG) (See figure 1a (1)) and stops at position 884/885/886 (TAA). the nucleic acid sequence encoding the urease Y subunit of *Helicobacter felis* strain CS1 starts at position 897/898/899 (ATG) and stops at position 2601/2602/2603 (TAG). Still even longer fragments, with a length of at least 55, 60 or 70 nucleic acids are in that order even more preferred.

[0009] A more preferred form of this embodiment relates to nucleic acid sequences having at least 90 %, preferably 94 %, more preferably 97 % homology with SEQ ID NO: 1.

[0010] The determination of the homology percentages was done with the computer program Align Plus for Windows, available from Scientific and Educational Software, P.O.Box 72045 Durham, NC 27722-2045, USA. Settings used for the nucleic acid comparisons are indicated in figures 1a, 1b and 1c.

[0011] Since the present invention discloses nucleic acid sequences encoding novel structural *Helicobacter felis* urease subunits, it is now for the first time possible to obtain such polypeptides in sufficient quantities. This can e.g. be done by using expression systems to express the genes encoding the UreX and UreY subunits.

Therefore, in a more preferred embodiment, the invention relates to DNA fragments comprising a nucleic acid sequence according to the invention. Such DNA fragments can e.g. be plasmids, into which a nucleic acid sequence according to the invention is cloned. Such DNA fragments are useful e.g. for enhancing the amount of DNA for use as a probe, as described below.

[0012] An essential requirement for the expression of the nucleic acid sequence is an adequate promoter operably linked to the nucleic acid sequence. It is obvious to those skilled in the art that the choice of a promoter extends to any

US005843460A

United States Patent [19]

Labigne et al.

[11] Patent Number: 5,843,460

[45] Date of Patent: Dec. 1, 1998

[54] **IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES**

[75] Inventors: **Agnes Labigne**, Bures S/Yvette, France; **Sebastin Suerbaum**, Bochum, Germany; **Richard L. Ferrero**, Paris; **Jean-Michel Thiberge**, Plaisir, both of France

[73] Assignees: **Institut Pasteur; Institut National de la Sante et de la Recherche Medicale**, both of Paris, France

[21] Appl. No.: **467,822**

[22] Filed: **Jun. 6, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 447,177, May 19, 1995, which is a continuation-in-part of Ser. No. 432,697, May 2, 1995.

[30] **Foreign Application Priority Data**

May 19, 1993 [EP] European Pat. Off. 93 401 309
Nov. 19, 1993 [WO] WIPO PCT/EP93/03259

[51] **Int. Cl.**[6] **A61K 39/02**

[52] **U.S. Cl.** **424/234.1**; 435/7.32; 435/6; 435/7.9; 514/234.5; 514/41

[58] **Field of Search** 435/7.32, 4, 6, 435/7.9; 514/234.5, 41; 424/234.1

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

WOA9004030	4/1990	WIPO .
WOA9109049	6/1991	WIPO .
WOA9307273	4/1993	WIPO .
WOA9316723	9/1993	WIPO .
WOA9318150	9/1993	WIPO .
WOA9320843	10/1993	WIPO .
WOA9406474	3/1994	WIPO .
WOA9409823	5/1994	WIPO .
WOA9503824	2/1995	WIPO .
WO 9638475	12/1996	WIPO .
WO 9640893	12/1996	WIPO .

OTHER PUBLICATIONS

R. L. Ferrero et al., "Molecular Evidence Demonstrating Significant Homology Between the Urease Polypeptides of Helicobacter Felis and and Helicobacter Pylori," *Gastroenterology*, vol. 104, No. 4, Apr. 1993, Elsevier, New York, U.S.; p. A699.

E.G. Fox, et al. "Comparison of Two New Immunodiagnostic Assays for Helicobacter Pylori with Established Clinical and Histopathologic Findings", Gastroeneterology, vol. 100, No. 5, Part 2, p. A66.

B.E. Dunn et al., "Identification and Purification of a cpn60 Heat Shock Protein Homolog from Helicobacter Pylori," *Infection and Immunity*, vol. 60, No. 5, May 1992, Am. Soc. Microbiol., Baltimore, US; pp. 1946–1951.

(List continued on next page.)

Primary Examiner—James C. Housel
Assistant Examiner—Ginny Allen Portner
Attorney, Agent, or Firm—Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.

[57] **ABSTRACT**

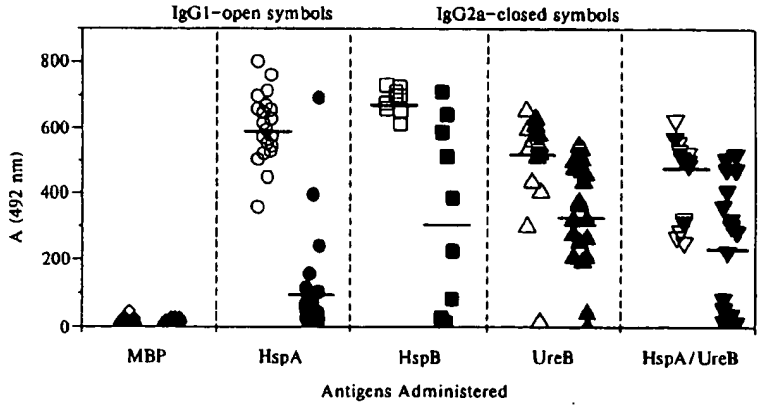
There is provided an immunogenic composition capable of inducing protective antibodies against Helicobacter infection characterized in that it comprises:

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NOS:20–21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NOS:20–21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NOS:22–26);

ii) and/or, a heat shock protein (Hsp), or chaperonin, from Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

10 Claims, 36 Drawing Sheets



==========

Welcome to the SIB BLAST Network Service

If results of this search are reported or published, please mention that the computation was performed at the SIB using the BLAST network service. The SIB BLAST network service uses a server developed at SIB and the NCBI BLAST 2 software.

In case of problems, please read the online BLAST help.
If your question is not covered, please contact <helpdesk@expasy.org>.

NCBI BLAST program reference [PMID:9254694]:
Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402(1997).

==========

Query: 243 AA (of which 10% low-complexity regions filtered out)
Date run: 2005-06-10 03:32:14 UTC+0100 on sib-gm1.unil.ch
Program: NCBI BLASTP 1.5.4-Paracel [2003-06-05]
Database: EXPASY/UniProtKB
1,974,938 sequences; 640,866,274 total letters
UniProt Release 5.2 consists of: Swiss-Prot Release 47.2 of 07-Jun-2005: 184304 en
TrEMBL Release 30.2 of 07-Jun-2005: 1779481 entrie

Taxonomic view | NiceBlast view | Printable view

List of potentially matching sequences

Send selected sequences to Clustal W (multiple alignment) Submit Query
Select up to...

☐ Include query sequence

	Db	AC	Description	Score	E-value
☐	sp	Q08716	URE2_HELFE Urease beta subunit (EC 3.5.1.5) (Urea amid...	437	e-122
☐	tr	Q9RGP5	_HELFE Urease (Fragment) [ureB] [Helicobacter felis]	437	e-122
☐	tr	Q8KT24	_HELFE Urease UreB (Fragment) [ureB] [Helicobacter felis]	437	e-122
☐	tr	Q8KT17	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	437	e-122
☐	tr	Q6UK80	_HELFE UreB (Fragment) [ureB] [Helicobacter felis]	437	e-122
☐	tr	Q6UK78	_HELFE UreB (Fragment) [ureB] [Helicobacter felis]	437	e-122
☐	tr	Q6UK66	_HELFE UreB (Fragment) [ureB] [Helicobacter felis]	437	e-122
☐	tr	Q6UK82	_HELFE UreB (Fragment) [ureB] [Helicobacter felis]	436	e-121
☐	tr	Q8KT28	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	435	e-121
☐	tr	Q8KT23	_9HELI Urease UreB (Fragment) [ureB] [Helicobacter salo...	434	e-120

☐	tr	Q6UK68	_9HELI UreB (Fragment) [ureB] [Helicobacter salomonis]	423 e-117
☐	tr	Q8KT25	_HELBI Urease UreB (Fragment) [ureB] [Helicobacter bizz...	413 e-114
☐	tr	Q8GH97	_HELBI Urease B [Helicobacter bizzozeronii]	413 e-114
☐	tr	Q9X753	_9HELI Urease (Fragment) [ureB] [Helicobacter salomonis]	409 e-113
☐	tr	Q8KT26	_HELBI Urease UreB (Fragment) [ureB] [Helicobacter bizz...	409 e-113
☐	tr	Q6UK76	_HELPY UreB (Fragment) [ureB] [Helicobacter pylori (Cam...	408 e-113
☐	tr	Q9S6R9	_9HELI Urease (Fragment) [ureB] [Helicobacter salomonis]	403 e-111
☐	sp	P42823	URE2_HELHE Urease beta subunit (EC 3.5.1.5) (Urea amid...	400 e-110
☐	tr	Q8KT16	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	400 e-110
☐	tr	Q8KT27	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	400 e-110
☐	tr	Q8KT31	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	398 e-110
☐	tr	Q8KT32	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	398 e-110
☐	tr	Q8KT22	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	398 e-110
☐	tr	Q8KT21	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	398 e-110
☐	tr	Q8KT18	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	398 e-110
☐	tr	Q8KT29	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	394 e-109
☐	tr	Q8KT19	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	392 e-108
☐	tr	Q8KT13	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	392 e-108
☐	tr	Q8KT20	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	389 e-107
☐	sp	P69996	URE2_HELPY Urease beta subunit (EC 3.5.1.5) (Urea amid...	378 e-104
☐	sp	P69997	URE2_HELPJ Urease beta subunit (EC 3.5.1.5) (Urea amid...	378 e-104
☐	tr	Q9S0Q5	_HELPY Urease B [ureB] [Helicobacter pylori (Campylobac...	378 e-104
☐	tr	Q8KT12	_HELPY Urease UreB (Fragment) [ureB] [Helicobacter pylo...	378 e-104
☐	tr	Q84F75	_HELPY Urease beta (EC 3.5.1.5) [ureB] [Helicobacter py...	378 e-104
☐	tr	Q7X3W5	_HELPY Urease B [ureB] [Helicobacter pylori (Campylobac...	378 e-104
☐	tr	Q6UK72	_HELPY UreB (Fragment) [ureB] [Helicobacter pylori (Cam...	378 e-104
☐	tr	Q64EY3	_HELPY UreB [ureB] [Helicobacter pylori (Campylobacter ...	378 e-104
☐	tr	Q9X742	_HELBI Urease (Fragment) [ureB] [Helicobacter bizzozero...	377 e-103
☐	tr	Q8KT33	_HELPY Urease UreB (Fragment) [ureB] [Helicobacter pylo...	377 e-103
☐	tr	Q8RNU6	_HELPY Urease B subunit [ureB] [Helicobacter pylori (Ca...	376 e-103
☐	tr	Q8KT30	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	376 e-103
☐	tr	Q8KT11	_9HELI Urease UreB (Fragment) [ureB] [Helicobacter sp. ...	376 e-103
☐	tr	Q9AFB1	_HELPY Urease B [Helicobacter pylori (Campylobacter pyl...	375 e-103
☐	tr	Q9S6R7	_HELBI Urease (Fragment) [ureB] [Helicobacter bizzozero...	373 e-102
☐	tr	Q6UK74	_HELPY UreB (Fragment) [ureB] [Helicobacter pylori (Cam...	372 e-102
☐	tr	Q6UK70	_HELPY UreB (Fragment) [ureB] [Helicobacter pylori (Cam...	372 e-102
☐	tr	Q8KT15	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	346 2e-94
☐	sp	P50045	URE2_HELMU Urease beta subunit (EC 3.5.1.5) (Urea amid...	323 1e-87
☐	tr	Q9R8R3	_HELHP Urease beta subunit (Fragment) [ureB] [Helicobac...	319 4e-86
☐	tr	Q9R8R1	_HELHP Urease beta subunit (Fragment) [ureB] [Helicobac...	317 1e-85
☐	tr	Q93PJ4	_HELHP Urease beta subunit UreB (EC 3.5.1.5) [ureB] [He...	317 2e-85
☐	tr	Q9ZHF1	_HELHP Urease beta subunit (Fragment) [ureB] [Helicobac...	317 2e-85
☐	tr	Q9RGP8	_9HELI Urease (Fragment) [ureB] [Flexispira rappini]	312 3e-84
☐	tr	Q8KT14	_HELHE Urease UreB (Fragment) [ureB] [Helicobacter heil...	311 6e-84
☐	tr	Q9RGP7	_HELHP Urease (Fragment) [ureB] [Helicobacter hepaticus]	311 1e-83

☐	tr Q9RGP6	_HELHP Urease (Fragment) [ureB] [Helicobacter hepaticus]	310	2e-83
☐	tr Q9RGQ0	_9HELI Urease (Fragment) [ureB] [Flexispira rappini]	308	5e-83
☐	tr Q9RGP9	_9HELI Urease (Fragment) [ureB] [Flexispira rappini]	308	5e-83
☐	sp Q07397	URE1_BACSB Urease alpha subunit (EC 3.5.1.5) (Urea ami...	295	4e-79
☐	tr Q9KG59	_BACHD Urease alpha subunit (EC 3.5.1.5) [ureC] [Bacill...	289	3e-77
☐	tr Q8GLB5	_HELHE UreB (Fragment) [Helicobacter heilmannii]	287	1e-76
☐	tr Q5KYM1	_GEOKA Urease alpha subunit (Urea amidohydrolase) (EC 3...	286	2e-76
☐	tr Q8GLB4	_HELHE UreB (Fragment) [Helicobacter heilmannii]	286	2e-76
☐	tr Q62HS0	_BURMA Urease, alpha subunit (EC 3.5.1.5) [ureC] [Burkh...	285	8e-76
☐	tr Q63RL3	_BURPS Urease alpha subunit (EC 3.5.1.5) [ureC] [Burkho...	285	8e-76
☐	tr Q5FB23	_CAMLA Urease B subunit [ureB] [Campylobacter lari]	285	8e-76
☐	tr Q8GLB3	_HELHE UreB (Fragment) [Helicobacter heilmannii]	283	2e-75
☐	tr Q8XXT1	_RALSO PROBABLE UREASE (ALPHA SUBUNIT) PROTEIN (EC 3.5....	282	4e-75
☐	tr Q8GLB6	_HELHE UreB (Fragment) [Helicobacter heilmannii]	282	5e-75
☐	tr Q8GLB2	_HELHE UreB (Fragment) [Helicobacter heilmannii]	281	6e-75
☐	tr Q8YQZ0	_ANASP Urease alpha subunit [alr3670] [Anabaena sp. (st...	281	1e-74
☐	tr Q6I6H5	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	280	2e-74
☐	tr Q6I6I9	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	278	5e-74
☐	tr Q6I6H3	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	278	5e-74
☐	tr Q6I6I3	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	278	7e-74
☐	tr Q7V3V2	_PROMM Urease alpha subunit (EC 3.5.1.5) [ureC] [Prochl...	277	1e-73
☐	tr Q6I6J1	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	277	1e-73
☐	tr Q6I6I5	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	277	2e-73
☐	tr Q6I6H1	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	276	2e-73
☐	tr Q5E728	_VIBF1 Urease alpha subunit (EC 3.5.1.5) [VF0673] [Vibr...	276	2e-73
☐	tr Q8DMV6	_SYNEL Urease alpha subunit [ureC] [Synechococcus elong...	276	4e-73
☐	tr Q4ZN06	_PSESY Urease (EC 3.5.1.5) [Psyr_4436] [Pseudomonas syr...	275	8e-73
☐	tr Q9RYJ4	_DEIRA Urease, alpha subunit [DRA0318] [Deinococcus rad...	274	1e-72
☐	tr Q87VP0	_PSESM Urease, alpha subunit [ureC] [Pseudomonas syring...	274	1e-72
☐	sp P16122	URE1_PROVU Urease alpha subunit (EC 3.5.1.5) (Urea ami...	274	1e-72
☐	tr Q733J6	_BACC1 Urease alpha subunit (EC 3.5.1.5) [ureC] [Bacill...	273	2e-72
☐	sp P17086	URE1_PROMI Urease alpha subunit (EC 3.5.1.5) (Urea ami...	273	3e-72
☐	sp P73061	URE1_SYNY3 Urease alpha subunit (EC 3.5.1.5) (Urea ami...	272	4e-72
☐	tr Q7U3I3	_SYNPX Urease alpha subunit (EC 3.5.1.5) [ureC] [Synech...	272	4e-72
☐	tr O52305	_SYNP2 Urease alpha subunit [ureC] [Synechococcus sp. (...	272	4e-72
☐	tr Q6I6H7	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	272	5e-72
☐	tr Q6I6I1	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	271	1e-71
☐	tr Q6I6H9	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	271	1e-71
☐	tr Q6FD83	_ACIAD Urease alpha subunit (EC 3.5.1.5) [ureC] [Acinet...	270	1e-71
☐	tr Q9HUU5	_PSEAE Urease alpha subunit [ureC] [Pseudomonas aerugin...	270	3e-71
☐	tr Q88J04	_PSEPK Urease, alpha subunit [ureC] [Pseudomonas putida...	270	3e-71
☐	tr Q7V1B6	_PROMP Urease alpha subunit (EC 3.5.1.5) [ureC] [Prochl...	268	6e-71
☐	tr Q9L644	_PROMA UreC [ureC] [Prochlorococcus marinus]	268	6e-71
☐	sp P77837	URE1_BACSU Urease alpha subunit (EC 3.5.1.5) (Urea ami...	268	7e-71
☐	tr Q6I6I8	_CAMLA Urease beta subunit (Fragment) [ureB] [Campyloba...	268	7e-71

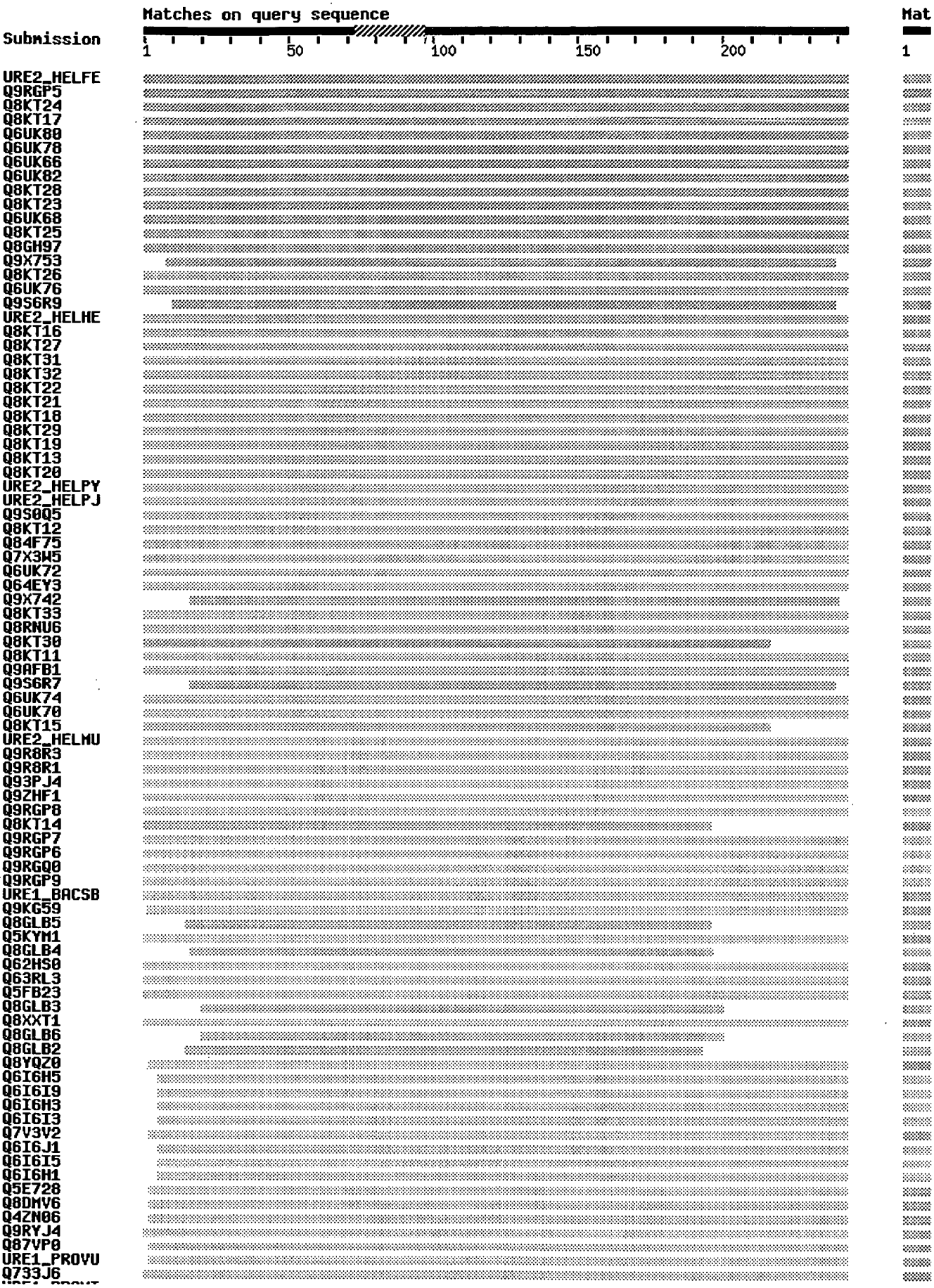
Graphical overview of the alignments

Click here to resubmit your query after masking regions matching PROSITE profiles or Pfam HMMs

(Help) (use ScanProsite for more details about PROSITE matches)

Profile hits

Pfam hits IMPDH


Matches on query sequence
Mat
Submission
1
50
100
150
200
1
URE2_HELFE
Q9RGP5
Q8KT24
Q8KT17
Q6UK80
Q6UK78
Q6UK66
Q6UK82
Q8KT28
Q8KT23
Q6UK68
Q8KT25
Q8GH97
Q9X753
Q8KT26
Q6UK76
Q9S6R9
URE2_HELHE
Q8KT16
Q8KT27
Q8KT31
Q8KT32
Q8KT22
Q8KT21
Q8KT18
Q8KT29
Q8KT19
Q8KT13
Q8KT20
URE2_HELPY
URE2_HELPJ
Q9S0Q5
Q8KT12
Q84F75
Q7X3W5
Q6UK72
Q64EY3
Q9X742
Q8KT33
Q8RNU6
Q8KT30
Q8KT11
Q9AFB1
Q9S6R7
Q6UK74
Q6UK70
Q8KT15
URE2_HELMU
Q9R8R3
Q9R8R1
Q93PJ4
Q9ZHF1
Q9RGP8
Q8KT14
Q9RGP7
Q9RGP6
Q9RGQ0
Q9RGP9
URE1_BACSB
Q9KG59
Q8GLB5
Q5KYM1
Q8GLB4
Q62HS0
Q63RL3
Q5FB23
Q8GLB3
Q8XXT1
Q8GLB6
Q8GLB2
Q8YQZ0
Q6I6H5
Q6I6I9
Q6I6H3
Q6I6I3
Q7V3V2
Q6I6J1
Q6I6I5
Q6I6H1
Q5E728
Q8DMV6
Q4ZN06
Q9RYJ4
Q87VP0
URE1_PROVU
Q733J6

Alignments

sp Q08716 URE2_HELFE **Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) [ureB] [Helicobacter felis]** 569 AA align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9RGP5 Q9RGP5_HELFE **Urease (Fragment) [ureB] [Helicobacter felis]** 243 AA align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 61  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 120

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240

Query: 241 IHT 243
           IHT
Sbjct: 241 IHT 243
```

tr Q8KT24 Q8KT24_HELFE **Urease UreB (Fragment) [ureB] [Helicobacter felis]** 384 AA align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT17 Q8KT17_HELHE **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 360 AA align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q6UK80 **UreB (Fragment) [ureB] [Helicobacter felis]** 360 AA
Q6UK80_HELFE align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q6UK78 **UreB (Fragment) [ureB] [Helicobacter felis]** 360 AA
Q6UK78_HELFE align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q6UK66 **UreB (Fragment) [ureB] [Helicobacter felis]** 362 AA
Q6UK66_HELFE align

Score = 437 bits (1124), Expect = e-122
Identities = 219/243 (90%), Positives = 219/243 (90%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q6UK82 **UreB (Fragment) [ureB] [Helicobacter felis]** 363 AA
Q6UK82_HELFE align

Score = 436 bits (1120), Expect = e-121
Identities = 218/243 (89%), Positives = 219/243 (89%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           E+YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EKYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT28 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 334 AA
Q8KT28_HELHE

align

Score = 435 bits (1118), Expect = e-121
Identities = 218/243 (89%), Positives = 218/243 (89%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIG KIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGLKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT23 **Urease UreB (Fragment) [ureB] [Helicobacter salomonis]** 359 AA
Q8KT23_9HELI

align

Score = 434 bits (1115), Expect = e-120
Identities = 217/243 (89%), Positives = 218/243 (89%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDT+LILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTNLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q6UK68 Q6UK68_9HELI **UreB (Fragment) [ureB] [Helicobacter salomonis]** 359 AA align

Score = 423 bits (1088), Expect = e-117
Identities = 213/243 (87%), Positives = 214/243 (87%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDT+LILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTNLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSML AA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSMLXAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT25 Q8KT25_HELBI **Urease UreB (Fragment) [ureB] [Helicobacter bizzozeronii]** 386 AA align

Score = 413 bits (1062), Expect = e-114
Identities = 205/243 (84%), Positives = 215/243 (88%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM+QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMAQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGV NNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDMQDGVCNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK+MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKTMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG+L KGNVSYEPSL DQ+EAGAIGFKIHEDWGSTPAAI+HCLNVAD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSYEPSLVDQLEAGAIGFKIHEDWGSTPAAIYHCLNVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8GH97 **Urease B [Helicobacter bizzozeronii]** 569 AA
Q8GH97_HELBI align

Score = 413 bits (1062), Expect = e-114
Identities = 205/243 (84%), Positives = 215/243 (88%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM+QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMAQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                        MQDGV NNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDMQDGVCNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK+MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKTMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG+L KGNVSYEPSL DQ+EAGAIGFKIHEDWGSTPAAI+HCLNVAD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSYEPSLVDQLEAGAIGFKIHEDWGSTPAAIYHCLNVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9X753 **Urease (Fragment) [ureB] [Helicobacter salomonis]** 231 AA
Q9X753_9HELI align

Score = 409 bits (1051), Expect = e-113
Identities = 205/231 (88%), Positives = 206/231 (88%)

```
Query: 9   PTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNAL 68
           PTTGDRVRLGDT+LILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNAL
Sbjct: 1   PTTGDRVRLGDTNLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNAL 60

Query: 69  IVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGID 128
           IVDYT                       MQDGVDNNLCVGPATEALAAEGLIVTAGGID
Sbjct: 61  IVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGLIVTAGGID 120

Query: 129 THIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLG 188
           THIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSMLRAAEEYAMNLG
Sbjct: 121 THIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSMLRAAEEYAMNLG 180

Query: 189 FLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           FLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV
Sbjct: 181 FLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 231
```

tr Q8KT26 **Urease UreB (Fragment) [ureB] [Helicobacter bizzozeronii]** 377 AA
Q8KT26_HELBI align

Score = 409 bits (1051), Expect = e-113

```
 Identities = 201/243 (82%), Positives = 213/243 (86%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLIL VEHDCTTYGEEIKFGGGKTIRDGM+QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILXVEHDCTTYGEEIKFGGGKTIRDGMAQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                         MQDGV NNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDMQDGVCNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK+MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKTMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
            EYAMNLG+L KGNVSYEPSL DQ+EAGAIGFKIHZDWGSTPAAIHHCLNVAB+YBVQVA
Sbjct: 187 XEYAMNLGYLGKGNVSYEPSLVDQLEAGAIGFKIHZDWGSTPAAIHHCLNVABKYBVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q6UK76 Q6UK76_HELPY **UreB (Fragment) [ureB] [Helicobacter pylori (Campylobacter pylori)]** 360 AA align

```
 Score =  408 bits (1048), Expect = e-113
 Identities = 202/243 (83%), Positives = 211/243 (86%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                         MQDGV NNLCVGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVAD+YDVQVA
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9S6R9 Q9S6R9_9HELI **Urease (Fragment) [ureB] [Helicobacter salomonis]** 231 AA align

```
 Score =  403 bits (1035), Expect = e-111
```

```
 Identities = 202/229 (88%), Positives = 204/229 (88%)

Query: 11  TGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIV 70
           +GDRVRLGDT+LILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIV
Sbjct: 3   SGDRVRLGDTNLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIV 62

Query: 71  DYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTH 130
           DYT                       MQDGVDNNLCVGPATEALAAEGLIVTAGGIDTH
Sbjct: 63  DYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTH 122

Query: 131 IHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFL 190
           IHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLKSMLRAAEEYAMNLGFL
Sbjct: 123 IHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRGNLKSMLRAAEEYAMNLGFL 182

Query: 191 AKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           AKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV
Sbjct: 183 AKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 231
```

sp P42823 URE2_HELHE **Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) [ureB] [Helicobacter heilmannii]** 568 AA align

```
 Score =  400 bits (1028), Expect = e-110
 Identities = 197/243 (81%), Positives = 211/243 (86%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                       +QDGV N LCVGPATEALAAEGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDLQDGVCNRLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG+L KGNVS+EP+L DQ+EAGAIGFKIHEDWGSTP+AI+H LN+AD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSFEPALIDQLEAGAIGFKIHEDWGSTPSAINHALNIADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT16 Q8KT16_HELHE **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 358 AA align

```
 Score =  400 bits (1028), Expect = e-110
 Identities = 197/243 (81%), Positives = 211/243 (86%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
```

```
            KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7    KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61   DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
            DLV+TNALIVDYT                          +QDGV N LCVGPATEALAAEGL
Sbjct: 67   DLVITNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDLQDGVCNRLCVGPATEALAAEGL 126

Query: 121  IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
            IVTAGGIDTHIHFISPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127  IVTAGGIDTHIHFISPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRAS 186

Query: 181  EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
            EEYAMNLG+L KGNVS+EP+L DQ+EAGAIGFKIHEDWGSTP+AI+H LN+AD+YDVQVA
Sbjct: 187  EEYAMNLGYLGKGNVSFEPALIDQLEAGAIGFKIHEDWGSTPSAINHALNIADKYDVQVA 246

Query: 241  IHT 243
            IHT
Sbjct: 247  IHT 249
```

tr Q8KT27 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 357 AA
Q8KT27_HELHE

align

```
 Score =  400 bits (1027), Expect = e-110
 Identities = 196/243 (80%), Positives = 209/243 (85%)

Query: 1    KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
            KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7    KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61   DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
            DLV+TNALIVDYT                          +QDGV N LCVGPATEALA EGL
Sbjct: 67   DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCNRLCVGPATEALAGEGL 126

Query: 121  IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
            IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127  IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGRWNLKEMLRAS 186

Query: 181  EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
            EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187  EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241  IHT 243
            IHT
Sbjct: 247  IHT 249
```

tr Q8KT31 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 355 AA
Q8KT31_HELHE

align

```
 Score =  398 bits (1023), Expect = e-110
 Identities = 196/243 (80%), Positives = 210/243 (85%)
```

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                        +QDGV N LCVGPATEALAAEGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDLQDGVCNRLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTMIGGGTGPADGTNA TITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMIGGGTGPADGTNAXTITPGRWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG+L KGNVS+EP+L DQ+EAGAIGFKIHEDWGSTP+AI+H LN+AD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSFEPALIDQLEAGAIGFKIHEDWGSTPSAINHALNIADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT32 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 358 AA
Q8KT32_HELHE

align

Score = 398 bits (1022), Expect = e-110
Identities = 195/243 (80%), Positives = 208/243 (85%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                        +QDGV N LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG  NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT22 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 356 AA
Q8KT22_HELHE

align

Score = 398 bits (1022), Expect = e-110
Identities = 195/243 (80%), Positives = 208/243 (85%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                        +QDGV N LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG  NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLIEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT21 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 358 AA
Q8KT21_HELHE

align

```
 Score =  398 bits (1022), Expect = e-110
 Identities = 195/243 (80%), Positives = 208/243 (85%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                        +QDGV N LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG  NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLIEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT18 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 357 AA
Q8KT18_HELHE

align

```
 Score =  398 bits (1022), Expect = e-110
```

```
 Identities = 195/243 (80%), Positives = 208/243 (85%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                        +QDGV N LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG  NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT29 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 379 AA
Q8KT29_HELHE

align

```
 Score =  394 bits (1013), Expect = e-109
 Identities = 195/243 (80%), Positives = 208/243 (85%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                        QDGV N LCVGPATEALAAEGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKNGKIHGIGKAGNKDXQDGVCNRLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG+L KGNVS+EP+  DQ+EAGAIG KIHEDWGSTP+AI+H LN+AD+YDVQVA
Sbjct: 187 EEYAMNLGYLGKGNVSFEPAXIDQLEAGAIGXKIHEDWGSTPSAINHALNIADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT19 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 357 AA
Q8KT19_HELHE

align

```
 Score =  392 bits (1006), Expect = e-108
 Identities = 193/243 (79%), Positives = 207/243 (84%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVE DCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVECDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                         +QDGV + LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCDRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG  NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT13 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 357 AA
Q8KT13_HELHE

align

```
 Score =  392 bits (1006), Expect = e-108
 Identities = 193/243 (79%), Positives = 207/243 (84%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVE DCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVECDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                         +QDGV + LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCDRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPG  NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT20 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 356 AA
Q8KT20_HELHE

align

```
 Score =  389 bits (1000), Expect = e-107
 Identities = 192/243 (79%), Positives = 206/243 (84%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVE DCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVECDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                        +QDGV + LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCDRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+T M+GGGTGPADGTNATTITPG  NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITPMLGGGTGPADGTNATTITPGHWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLG++ KGNVSYEPSL +Q+EAGAIGFKIHEDWGSTP+AIHH L +ADEYDVQVA
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGFKIHEDWGSTPSAIHHALKIADEYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

sp P69996 URE2_HELPY **Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) [ureB] [Helicobacter pylori (Campylobacter pylori)]** 569 AA align

```
 Score =  378 bits (970), Expect = e-104
 Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

sp P69997 URE2_HELPJ **Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) [ureB] [Helicobacter pylori J99 (Campylobacter pylori J99)]** 569 AA align

```
 Score =  378 bits (970), Expect = e-104
 Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9S0Q5 Q9S0Q5_HELPY **Urease B [ureB] [Helicobacter pylori (Campylobacter pylori)]** 569 AA align

```
 Score =  378 bits (970), Expect = e-104
 Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT12 Q8KT12_HELPY **Urease UreB (Fragment) [ureB] [Helicobacter pylori SS1]** 378 AA align

```
 Score =  378 bits (970), Expect = e-104
 Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q84F75 Q84F75_HELPY **Urease beta (EC 3.5.1.5) [ureB] [Helicobacter pylori (Campylobacter pylori)]** 569 AA align

```
 Score =  378 bits (970), Expect = e-104
 Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q7X3W5 Q7X3W5_HELPY **Urease B [ureB] [Helicobacter pylori (Campylobacter pylori)]** 569 AA align

```
 Score =  378 bits (970), Expect = e-104
 Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q6UK72 Q6UK72_HELPY **UreB (Fragment) [ureB] [Helicobacter pylori (Campylobacter pylori)]** 370 AA align

```
 Score =  378 bits (970), Expect = e-104
 Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q64EY3 Q64EY3_HELPY **UreB [ureB] [Helicobacter pylori (Campylobacter pylori)]** 567 AA align

```
 Score =  378 bits (970), Expect = e-104
 Identities = 189/243 (77%), Positives = 204/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 5   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 64

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 65  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 124

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 125 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 184

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 185 EEYSMNLGFLAKGNTSNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 244

Query: 241 IHT 243
           IHT
Sbjct: 245 IHT 247
```

tr Q9X742 Q9X742_HELBI **Urease (Fragment) [ureB] [Helicobacter bizzozeronii]** 231 AA

align

```
 Score =  377 bits (968), Expect = e-103
 Identities = 188/224 (83%), Positives = 196/224 (86%)

Query: 17  LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXXX 76
           LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM+QTNSPSS+ELDLVLTNALIVDYT
Sbjct: 8   LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMAQTNSPSSHELDLVLTNALIVDYTGIY 67

Query: 77  XXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTHIHFISP 136
                               MQDGV NNLCVGPATEALAAEGLIVTAGGIDTHIHFISP
Sbjct: 68  KADIGIKNGKIHGIGKAGNKDMQDGVCNNLCVGPATEALAAEGLIVTAGGIDTHIHFISP 127

Query: 137 QQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVS 196
           QQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK+MLRA+EEYAMNLG+L KGNVS
Sbjct: 128 QQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKTMLRASEEYAMNLGYLGKGNVS 187

Query: 197 YEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           YEPSL DQ+EAGAIGFKIHEDWGSTPAAIHHCLNVAD+YDVQVA
Sbjct: 188 YEPSLVDQLEAGAIGFKIHEDWGSTPAAIHHCLNVADKYDVQVA 231
```

tr Q8KT33 Q8KT33_HELPY **Urease UreB (Fragment) [ureB] [Helicobacter pylori 26695]** 377 AA

align

```
 Score =  377 bits (968), Expect = e-103
 Identities = 189/243 (77%), Positives = 204/243 (83%)
```

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLVDQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8RNU6 Q8RNU6_HELPY **Urease B subunit [ureB] [Helicobacter pylori (Campylobacter pylori)]** 559 AA align

```
 Score =  376 bits (966), Expect = e-103
 Identities = 188/243 (77%), Positives = 203/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEY SMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYASMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNLGFLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT30 Q8KT30_HELHE **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 222 AA align

```
 Score =  376 bits (966), Expect = e-103
 Identities = 191/216 (88%), Positives = 191/216 (88%)
```

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL
Sbjct: 7   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                         MQDGVDNNLCVGPATEALAAEGL
Sbjct: 67  DLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDNNLCVGPATEALAAEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHE 216
           EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIG KIHE
Sbjct: 187 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGLKIHE 222
```

tr Q8KT11 **Urease UreB (Fragment) [ureB] [Helicobacter sp. TD1]** 387 AA
Q8KT11_9HELI

align

```
 Score =  376 bits (965), Expect = e-103
 Identities = 189/243 (77%), Positives = 203/243 (82%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                         MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHI FISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIXFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEYAMNLGFL KGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYAMNLGFLGKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9AFB1 **Urease B [Helicobacter pylori (Campylobacter pylori)]** 569 AA
Q9AFB1_HELPY

align

```
 Score =  375 bits (964), Expect = e-103
 Identities = 188/243 (77%), Positives = 203/243 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
```

```
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MNL FLAKGN S + SL DQIEAGAIGFKIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNLSFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9S6R7 Q9S6R7_HELBI **Urease (Fragment) [ureB] [Helicobacter bizzozeronii]** 230 AA

align

```
 Score =  373 bits (958), Expect = e-102
 Identities = 186/223 (83%), Positives = 195/223 (87%)

Query: 17  LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXXX 76
           LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM+QTNSPSS+ELDLVLTNALIVDYT
Sbjct: 8   LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMAQTNSPSSHELDLVLTNALIVDYTGIY 67

Query: 77  XXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTHIHFISP 136
                               MQDGV NNLCVGPATEALAAEGLIVTAGGIDTHIHFISP
Sbjct: 68  KADIGIKNGKIHGIGKAGNKDMQDGVCNNLCVGPATEALAAEGLIVTAGGIDTHIHFISP 127

Query: 137 QQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVS 196
           QQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK+MLRA+EEYAMNLG+L KGNVS
Sbjct: 128 QQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKTMLRASEEYAMNLGYLGKGNVS 187

Query: 197 YEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           YEPSL DQ+EAGAIGFKIHEDWGSTPAAI+HCLNVAD+YDVQV
Sbjct: 188 YEPSLVDQLEAGAIGFKIHEDWGSTPAAIYHCLNVADKYDVQV 230
```

tr Q6UK74 Q6UK74_HELPY **UreB (Fragment) [ureB] [Helicobacter pylori (Campylobacter pylori)]** 359 AA

align

```
 Score =  372 bits (955), Expect = e-102
 Identities = 186/243 (76%), Positives = 201/243 (82%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEY SMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYASMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
```

```
          DL++TNALIVDYT                                MQDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKFMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MN GFLAKGN S + SL DQIEAGAIG KIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNFGFLAKGNXSNDASLADQIEAGAIGXKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q6UK70 Q6UK70_HELPY **UreB (Fragment) [ureB] [Helicobacter pylori (Campylobacter pylori)]** 374 AA align

```
 Score =  372 bits (955), Expect = e-102
 Identities = 186/243 (76%), Positives = 201/243 (82%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLI EVEHD T YGEE+KFGGGKT+R+GMSQ+N+PS  EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKEEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DL++TNALIVDYT                        QDGV NNL VGPATEALA EGL
Sbjct: 67  DLIITNALIVDYTGIYKADIGIKDGKIAGIGKGGNKDTQDGVKNNLSVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGR NLK MLRAA
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKFMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY+MN GFLAKGN S + SL DQIEAGAIG KIHEDWG+TP+AI+H L+VAD+YDVQVA
Sbjct: 187 EEYSMNFGFLAKGNASNDASLADQIEAGAIGLKIHEDWGTTPSAINHALDVADKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q8KT15 Q8KT15_HELHE **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 222 AA align

```
 Score =  346 bits (888), Expect = 2e-94
 Identities = 172/216 (79%), Positives = 183/216 (84%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66
```

```
Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                          +QDGV N LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGRWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHE 216
           EEYAMNLG++ KGNVSYEPSL +Q+EAGAIG KIHE
Sbjct: 187 EEYAMNLGYMGKGNVSYEPSLVEQLEAGAIGLKIHE 222
```

sp P50045 URE2_HELMU **Urease beta subunit (EC 3.5.1.5) (Urea amidohydrolase) (Fragment) [ureB] [Helicobacter mustelae]** 308 AA align

```
 Score =  323 bits (829), Expect = 1e-87
 Identities = 163/243 (67%), Positives = 185/243 (76%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDT+LI E+E D T YGEEIKFGGGKTIRDGMSQ+ SP   EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTELIAEIEKDYTVYGEEIKFGGGKTIRDGMSQSVSPDVNEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                          QDGV  +L VG +TEA+A EGL
Sbjct: 67  DAVITNAMIIDYTGIYKADIGIKDGKIAGIGKAGNRDTQDGVGMDLVVGASTEAIAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISP QIPTA  SGVTTMIGGGTGPA GT ATTI+PG  N+K M+RAA
Sbjct: 127 IVTAGGIDTHIHFISPTQIPTALYSGVTTMIGGGTGPAAGTFATTISPGEWNIKQMIRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           EEY MNLGF  KGN S   +L DQI+AGA+GFK+HED GSTPA I+H L++A++YDVQVA
Sbjct: 187 EEYTMNLGFFGKGNTSNVKALEDQIKAGALGFKVHEDCGSTPAVINHSLDIAEKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9R8R3 Q9R8R3_HELHP **Urease beta subunit (Fragment) [ureB] [Helicobacter hepaticus]** 315 AA align

```
 Score =  319 bits (817), Expect = 4e-86
 Identities = 159/243 (65%), Positives = 181/243 (74%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           K+Y SMYGPTTGD+VRLGDT+L  E+E D T YGEEIKFGGGKTIRDGM+Q+ S  + EL
Sbjct: 7   KQYASMYGPTTGDKVRLGDTNLFAEIEKDYTLYGEEIKFGGGKTIRDGMAQSASTYTNEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                          QDGV+  + VG ATE +A EG
Sbjct: 67  DAVITNAMIIDYTGIYKADIGIKGGKIVGIGKAGNPDTQDGVNEAMVVGAATEVIAGEGQ 126
```

```
Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           I+TAGGIDTHIHFISP QIPTA SGVTTMIGGGTGPA GTNATT TPG+ N+ MLRAA
Sbjct: 127 IITAGGIDTHIHFISPTQIPTALYSGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           E YAMNLGF KGN S E L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQVA
Sbjct: 187 ESYAMNLGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9R8R1 Q9R8R1_HELHP **Urease beta subunit (Fragment) [ureB] [Helicobacter hepaticus]** 315 AA align

```
 Score =  317 bits (812), Expect = 1e-85
 Identities = 158/243 (65%), Positives = 180/243 (74%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           K+Y SMYGPTTGD+VRLGDT+L E+E D T YGEEIKFGGGKTIRDGM+Q+ S + EL
Sbjct: 7   KQYASMYGPTTGDKVRLGDTNLFAEIEKDYTLYGEEIKFGGGKTIRDGMAQSASTYTNEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                        QDGV+  + VG ATE +A EG
Sbjct: 67  DAVITNAMIIDYTGIYKADIGIKGGKIVGIGKAGNPDTQDGVNEAMVVGAATEVIAGEGQ 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           I+TAGGIDTHIHFISP QIPTA SGVTTMIGGGTGPA GTNATT TPG+ N+ MLRAA
Sbjct: 127 IITAGGIDTHIHFISPTQIPTALYSGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           E YAMNLGF KGN S E L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQV
Sbjct: 187 ESYAMNLGFFGKGNSSNEEGLXEQIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQVV 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q93PJ4 Q93PJ4_HELHP **Urease beta subunit UreB (EC 3.5.1.5) [ureB] [Helicobacter hepaticus]** 569 AA align

```
 Score =  317 bits (811), Expect = 2e-85
 Identities = 158/243 (65%), Positives = 180/243 (74%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           K+Y SMYGPTTGD+VRLGDT+L E+E D T YGEEIKFGGGKTIRDGM+Q+ S + EL
Sbjct: 7   KQYASMYGPTTGDKVRLGDTNLFAEIEKDYTLYGEEIKFGGGKTIRDGMAQSASTYTNEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                        QD V+  + VG ATE +A EG
```

```
Sbjct: 67  DAVITNAMIIDYTGIYKADIGIKGGKIVGIGKAGNPDTQDSVNEAMVVGAATEVIAGEGQ 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           I+TAGGIDTHIHFISP QIPTA  SGVTTMIGGGTGPA GTNATT TPG+ N+  MLRAA
Sbjct: 127 IITAGGIDTHIHFISPTQIPTALYSGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           E YAMNLGF  KGN S E  L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQVA
Sbjct: 187 ESYAMNLGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9ZHF1 Q9ZHF1_HELHP **Urease beta subunit (Fragment) [ureB] [Helicobacter hepaticus]** 315 AA align

```
 Score =  317 bits (811), Expect = 2e-85
 Identities = 158/243 (65%), Positives = 180/243 (74%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           K+Y SMYGPTTGD+VRLGDT+L  E+E D T YGEEIKFGGGKTIRDGM+Q+ S  + EL
Sbjct: 7   KQYASMYGPTTGDKVRLGDTNLFAEIEKDYTLYGEEIKFGGGKTIRDGMAQSASTYTNEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                        QD V+  + VG ATE +A EG
Sbjct: 67  DAVITNAMIIDYTGIYKADIGIKGGKIVGIGKAGNPDTQDSVNEAMVVGAATEVIAGEGQ 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           I+TAGGIDTHIHFISP QIPTA  SGVTTMIGGGTGPA GTNATT TPG+ N+  MLRAA
Sbjct: 127 IITAGGIDTHIHFISPTQIPTALYSGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA 186

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           E YAMNLGF  KGN S E  L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQVA
Sbjct: 187 ESYAMNLGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQVA 246

Query: 241 IHT 243
           IHT
Sbjct: 247 IHT 249
```

tr Q9RGP8 Q9RGP8_9HELI **Urease (Fragment) [ureB] [Flexispira rappini]** 243 AA align

```
 Score =  312 bits (800), Expect = 3e-84
 Identities = 155/243 (63%), Positives = 180/243 (73%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD++RLGDT+L  E+E D   YGEEIKFGGGKTIRDGM+Q+ S S  EL
Sbjct: 1   KEYVSMYGPTTGDKIRLGDTELFAEIEKDYAIYGEEIKFGGGKTIRDGMAQSVSDSENEL 60

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                        QDGV + L VG  TE +A EGL
```

```
Sbjct: 61  DSVITNAVIIDYTGIYKADIGIKNGKIFGIGKAGNKDTQDGVCDKLIVGTNTEVIAGEGL 120

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIH+ISP QIPTA  SGVTTMIGGGTGPA GT+ATT TPG  +++ M+RA
Sbjct: 121 IVTAGGIDTHIHYISPTQIPTALYSGVTTMIGGGTGPAAGTSATTCTPGSWHMREMIRAT 180

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           + YAMN GF  KGN S E +L  QIE+GA+G K+HEDWGSTPAAI+H LN+AD+YDVQVA
Sbjct: 181 QHYAMNFGFFGKGNSSNENALSKQIESGALGLKVHEDWGSTPAAINHALNIADKYDVQVA 240

Query: 241 IHT 243
           IHT
Sbjct: 241 IHT 243
```

tr Q8KT14 **Urease UreB (Fragment) [ureB] [Helicobacter heilmannii]** 202 AA
Q8KT14_HELHE

align

```
 Score =  311 bits (798), Expect = 6e-84
 Identities = 155/196 (79%), Positives = 164/196 (83%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD+VRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+EL
Sbjct: 7   KEYVSMYGPTTGDKVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHEL 66

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLV+TNALIVDYT                        +QDGV N LCVGPATEALA EGL
Sbjct: 67  DLVITNALIVDYTGIYKADIGIKDGKIHGIGKAGNKDIQDGVCNRLCVGPATEALAGEGL 126

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQIPTAFASG+TTM+GGGTGPADGTNATTITPGR NLK MLRA+
Sbjct: 127 IVTAGGIDTHIHFISPQQIPTAFASGITTMLGGGTGPADGTNATTITPGRWNLKEMLRAS 186

Query: 181 EEYAMNLGFLAKGNVS 196
           E YAMNLG++ KGNVS
Sbjct: 187 EXYAMNLGYMGKGNVS 202
```

tr Q9RGP7 **Urease (Fragment) [ureB] [Helicobacter hepaticus]** 243 AA
Q9RGP7_HELHP

align

```
 Score =  311 bits (796), Expect = 1e-83
 Identities = 156/243 (64%), Positives = 177/243 (72%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEY SMY PTTGD+VRLGDT+L  E+E D T YGEEIKFGGGKTIRDGM+Q+    + EL
Sbjct: 1   KEYASMYAPTTGDKVRLGDTNLFAEIEKDYTLYGEEIKFGGGKTIRDGMAQSAXTYTNEL 60

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                        QD V+  + VG ATE +A EG
Sbjct: 61  DAVITNAMIIDYTGIYKADIGIKGGKIVGIGKAGNPDTQDSVNEAMVVGAATEVIAGEGX 120

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           I+TAGGIDTHIHFISP QIPTA  SGVTTMIGG TGPA GTNATT TPG+ N+  MLRAA
```

```
Sbjct: 121 IITAGGIDTHIHFISPTQIPTAXYSGVTTMIGGXTGPAAGTNATTCTPGKWNMHQMLRAA 180

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           E YAMNLGF  KGN S E  L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQVA
Sbjct: 181 ESYAMNLGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAXKYDVQVA 240

Query: 241 IHT 243
           IHT
Sbjct: 241 IHT 243
```

tr Q9RGP6 Q9RGP6_HELHP **Urease (Fragment) [ureB] [Helicobacter hepaticus]** 243 AA align

```
 Score =  310 bits (793), Expect = 2e-83
 Identities = 155/243 (63%), Positives = 176/243 (71%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEY SMYGPTTGD+VRLGDT+L  E+E D T YGEEIKFGGGKTIRDGM+Q+     + EL
Sbjct: 1   KEYASMYGPTTGDKVRLGDTNLFAEIEKDYTLYGEEIKFGGGKTIRDGMAQSAXTYTNEL 60

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                          QD V+  + VG ATE +A EG
Sbjct: 61  DAVITNAMIIDYTGIYKADIGIKGGKIVGIGKAGNPDTQDSVNEAMVVGAATEVIAGEGQ 120

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           I+TAGGIDTHIHFISP QIPTA  SGVTTMIGGGTGPA GTNATT TPG+ N+  MLRAA
Sbjct: 121 IITAGGIDTHIHFISPTQIPTALYSGVTTMIGGGTGPAAGTNATTCTPGKWNMHQMLRAA 180

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           E YAMN GF  KGN S E  L +QI+AGA+G K+HEDWGSTPAAI+H LNVA +YDVQ A
Sbjct: 181 ESYAMNQGFFGKGNSSNEEGLEEQIKAGALGLKVHEDWGSTPAAINHALNVAQKYDVQCA 240

Query: 241 IHT 243
            HT
Sbjct: 241 SHT 243
```

tr Q9RGQ0 Q9RGQ0_9HELI **Urease (Fragment) [ureB] [Flexispira rappini]** 243 AA align

```
 Score =  308 bits (790), Expect = 5e-83
 Identities = 153/243 (62%), Positives = 178/243 (72%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEY SMYGPTTGD++RLGDT+L  E+E D   YGEEIKFGGGKTIRDGM+Q+ S S  EL
Sbjct: 1   KEYXSMYGPTTGDKIRLGDTELFAEIEKDYAIYGEEIKFGGGKTIRDGMAQSVSDSENEL 60

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                          QDGV + L VG  TE +A EGL
Sbjct: 61  DSVITNAVIIDYTGIYKADIGIKNGKIFGIGKAGNKDTQDGVCDKLIVGTNTEVIAGEGL 120

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
            VTAGGIDTHIH+ISP QIPTA  SGVTTMIGGGTGPA GT+ATT TPG  +++ M+RA
Sbjct: 121 XVTAGGIDTHIHYISPTQIPTALYSGVTTMIGGGTGPAAGTSATTCTPGSWHMREMIRAT 180
```

```
Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           + YAMN GF  KGN S E +L  QIE+GA+G K+HEDWGSTPAAI+H LN+AD+YDVQVA
Sbjct: 181 QHYAMNFGFFGKGNSSNENALSKQIESGALGLKVHEDWGSTPAAINHALNIADKYDVQVA 240

Query: 241 IHT 243
           IHT
Sbjct: 241 IHT 243
```

tr Q9RGP9 Q9RGP9_9HELI **Urease (Fragment) [ureB] [Flexispira rappini]** 243 AA align

```
 Score =  308 bits (790), Expect = 5e-83
 Identities = 153/243 (62%), Positives = 179/243 (72%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           KEYVSMYGPTTGD++RLGDT+L  E+E D   YGEE+KFGGGKTIRDGM+Q+ S S  EL
Sbjct: 1   KEYVSMYGPTTGDKIRLGDTELFAEIEKDYAIYGEEVKFGGGKTIRDGMAQSVSDSENEL 60

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+DYT                        QDGV + L VG  TE +A EGL
Sbjct: 61  DSVITNAVIIDYTGIYKADIGIKNGKIFGIGKAGNKDTQDGVCDKLIVGTNTEVIAGEGL 120

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIH+ISP QIPTA SGVTTMIGGGTGPA GT+ATT TPG  +++ M+RA
Sbjct: 121 IVTAGGIDTHIHYISPTQIPTALYSGVTTMIGGGTGPAAGTSATTCTPGSWHMREMIRAT 180

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           + YAMN GF  KGN S E +L  QIE+GA+G K+HEDWGSTPAAI+H L+ AD+YDVQVA
Sbjct: 181 QHYAMNFGFFGKGNSSNENALSKQIESGALGLKVHEDWGSTPAAINHALSXADKYDVQVA 240

Query: 241 IHT 243
           IHT
Sbjct: 241 IHT 243
```

sp Q07397 URE1_BACSB **Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) [ureC] [Bacillus sp. (strain TB-90)]** 569 AA align

```
 Score =  295 bits (756), Expect = 4e-79
 Identities = 151/244 (61%), Positives = 176/244 (71%), Gaps = 3/244 (1%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYE- 59
           K+Y  M+GPT GD +RL D++L +E+E D TTYG+E+KFGGGK IRDGM Q    +S E
Sbjct: 8   KQYADMFGPTVGDAIRLADSELFIEIEKDYTTYGDEVKFGGGKVIRDGMGQHPLATSDEC 67

Query: 60  LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEG 119
           +DLVLTNA+IVDYT                       + DGVD  + +G ATE +AAEG
Sbjct: 68  VDLVLTNAIIVDYTGIYKADIGIKDGMIASIGKAGNPLLMDGVD--MVIGAATEVIAAEG 125

Query: 120 LIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRA 179
           +IVTAGGID HIHFI PQQI TA ASGVTTMIGGGTGPA GTNATT TPG  N+  ML+A
Sbjct: 126 MIVTAGGIDAHIHFICPQQIETALASGVTTMIGGGTGPATGTNATTCTPGPWNIHRMLQA 185
```

```
Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           AEE+ +NLGFL KGN S E  L++QIEAGA+G K+HEDWGST AAI  CL VAD YDVQV
Sbjct: 186 AEEFPINLGFLGKGNCSDEAPLKEQIEAGAVGLKLHEDWGSTAAAIDTCLKVADRYDVQV 245

Query: 240 AIHT 243
           AIHT
Sbjct: 246 AIHT 249
```

tr Q9KG59 Q9KG59_BACHD **Urease alpha subunit (EC 3.5.1.5) [ureC] [Bacillus halodurans]** 571 AA align

```
 Score =  289 bits (740), Expect = 3e-77
 Identities = 144/244 (59%), Positives = 176/244 (72%), Gaps = 2/244 (0%)

Query: 2   EYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYE-- 59
           ++ S+YGPT GD+VRL DTDL+LE+E D T YG+E KFGGGK +RDGM Q+    +  E
Sbjct: 7   QHASLYGPTVGDKVRLADTDLLLEIEKDYTVYGDECKFGGGKVLRDGMGQSAVYTRDEGV 66

Query: 60  LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEG 119
           LDL++TNA I+DYT                        + DGV++++ VG +TEA+A EG
Sbjct: 67  LDLIITNATIIDYTGIVKADIGIKDGHIVGIGKGGNPDIMDGVESHMIVGASTEAIAGEG 126

Query: 120 LIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRA 179
           LIVTAGGID HIHFISPQQI  A ASG+TTM+GGGTGPA GT ATT TPG+ N++ ML A
Sbjct: 127 LIVTAGGIDAHIHFISPQQIDVAIASGITTMLGGGTGPATGTKATTCTPGKWNIERMLEA 186

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           A+ + +NLGFL KGN S    LR+QIEAGAIG K+HEDWG+TPAAI  CL+VAD  DVQV
Sbjct: 187 ADAFPVNLGFLGKGNASTPAPLREQIEAGAIGLKLHEDWGTTPAAIRTCLSVADRMDVQV 246

Query: 240 AIHT 243
           AIHT
Sbjct: 247 AIHT 250
```

tr Q8GLB5 Q8GLB5_HELHE **UreB (Fragment) [Helicobacter heilmannii]** 181 AA align

```
 Score =  287 bits (735), Expect = 1e-76
 Identities = 145/181 (80%), Positives = 151/181 (83%)

Query: 16  RLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXX 75
           RLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+ELDLV+TNALIVDYT
Sbjct: 1   RLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHELDLVITNALIVDYTGI 60

Query: 76  XXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTHIHFIS 135
                                +QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFIS
Sbjct: 61  YKADIGIKNGKIHGIGKAGNKDLQDGVCNRLCVGPATEALAAEGLIVTAGGIDTHIHFIS 120

Query: 136 PQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNV 195
           PQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+EEYAMNLG+L KGNV
Sbjct: 121 PQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGKGNV 180
```

```
Query: 196 S 196
           S
Sbjct: 181 S 181
```

tr Q5KYM1 Q5KYM1_GEOKA **Urease alpha subunit (Urea amidohydrolase) (EC 3.5.1.5) [ureC] [Geobacillus kaustophilus]** 569 AA align

```
 Score =  286 bits (733), Expect = 2e-76
 Identities = 144/244 (59%), Positives = 172/244 (70%), Gaps = 3/244 (1%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYE- 59
           ++Y  M+GPTTGD +RL DTDL +E+EHD T YG+E+KFGGGK IRDGM Q    +  E
Sbjct: 8   RQYADMFGPTTGDCIRLADTDLWIEIEHDYTVYGDEVKFGGGKVIRDGMGQHPLATRDEA 67

Query: 60  LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEG 119
           +DLVLTNA+IVDYT                          + DGV  N+ +G +TE +AAEG
Sbjct: 68  VDLVLTNAVIVDYTGIYKADIGIKDGNIAAIGKAGNPLLMDGV--NIVIGASTEVIAAEG 125

Query: 120 LIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRA 179
            IVTAGG+D HIHFI PQQI TA +SG+TTMIGGGTGPA GTNATT TPG  N+  ML A
Sbjct: 126 KIVTAGGVDAHIHFICPQQIETALSSGITTMIGGGTGPATGTNATTCTPGEWNIYRMLEA 185

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           AE + MN+GFL KGN S +  + +Q+ AGAIG K+HEDWG+T AAI  CL VADEYDVQV
Sbjct: 186 AEAFPMNIGFLGKGNASAKEPIAEQVRAGAIGLKLHEDWGTTAAAIDACLRVADEYDVQV 245

Query: 240 AIHT 243
           AIHT
Sbjct: 246 AIHT 249
```

tr Q8GLB4 Q8GLB4_HELHE **UreB (Fragment) [Helicobacter heilmannii]** 181 AA align

```
 Score =  286 bits (733), Expect = 2e-76
 Identities = 144/181 (79%), Positives = 151/181 (82%)

Query: 17  LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXXX 76
           LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+ELDLV+TNALIVDYT
Sbjct: 1   LGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHELDLVITNALIVDYTGIY 60

Query: 77  XXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTHIHFISP 136
                               +QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFISP
Sbjct: 61  KADIGIKNGKIHGIGKAGNKDLQDGVCNRLCVGPATEALAAEGLIVTAGGIDTHIHFISP 120

Query: 137 QQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVS 196
           QQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+EEYAMNLG+L KGNVS
Sbjct: 121 QQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGKGNVS 180

Query: 197 Y 197
           +
Sbjct: 181 F 181
```

tr Q62HS0 Q62HS0_BURMA **Urease, alpha subunit (EC 3.5.1.5) [ureC] [Burkholderia mallei (Pseudomonas mallei)]** 568 AA align

Score = 285 bits (728), Expect = 8e-76
Identities = 144/243 (59%), Positives = 173/243 (70%), Gaps = 2/243 (0%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           + Y  MYGPTTGDR+RL DT+L++EVE D T YGEE+KFGGGK IRDGM Q+  P++
Sbjct: 8   RAYAEMYGPTTGDRIRLADTELLIEVERDHTLYGEEVKFGGGKVIRDGMGQSQLPAADVA 67

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+D+                              +Q GV   + +G ATE +A EGL
Sbjct: 68  DTVITNAVILDHWGIVKADIAIKHGRIAAIGKAGNPDIQPGV--TIAIGAATEIIAGEGL 125

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQI  A ASGVTTMIGGGTGPA GTNATT TPG  +++ ML+AA
Sbjct: 126 IVTAGGIDTHIHFISPQQIDEALASGVTTMIGGGTGPATGTNATTCTPGPWHMERMLQAA 185

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           + + +NLGFL KGN S    L +QIEAGAIG K+HEDWG+TPAAI +CL VAD+ D QVA
Sbjct: 186 DGWPINLGFLGKGNASRPQPLVEQIEAGAIGLKLHEDWGTTPAAIDNCLTVADDTDTQVA 245

Query: 241 IHT 243
           IHT
Sbjct: 246 IHT 248
```

tr Q63RL3 Q63RL3_BURPS **Urease alpha subunit (EC 3.5.1.5) [ureC] [Burkholderia pseudomallei (Pseudomonas pseudomallei)]** 568 AA align

Score = 285 bits (728), Expect = 8e-76
Identities = 144/243 (59%), Positives = 173/243 (70%), Gaps = 2/243 (0%)

```
Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           + Y  MYGPTTGDR+RL DT+L++EVE D T YGEE+KFGGGK IRDGM Q+  P++
Sbjct: 8   RAYAEMYGPTTGDRIRLADTELLIEVERDHTLYGEEVKFGGGKVIRDGMGQSQLPAADVA 67

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNA+I+D+                              +Q GV   + +G ATE +A EGL
Sbjct: 68  DTVITNAVILDHWGIVKADIAIKHGRIAAIGKAGNPDIQPGV--TIAIGAATEIIAGEGL 125

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISPQQI  A ASGVTTMIGGGTGPA GTNATT TPG  +++ ML+AA
Sbjct: 126 IVTAGGIDTHIHFISPQQIDEALASGVTTMIGGGTGPATGTNATTCTPGPWHMERMLQAA 185

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           + + +NLGFL KGN S    L +QIEAGAIG K+HEDWG+TPAAI +CL VAD+ D QVA
Sbjct: 186 DGWPINLGFLGKGNASRPQPLVEQIEAGAIGLKLHEDWGTTPAAIDNCLTVADDTDTQVA 245

Query: 241 IHT 243
           IHT
```

```
Sbjct: 246 IHT 248
```

tr Q5FB23 **Urease B subunit [ureB] [Campylobacter lari]** 565 AA
Q5FB23_CAMLA align

```
 Score =  285 bits (728), Expect = 8e-76
 Identities = 147/243 (60%), Positives = 172/243 (70%), Gaps = 1/243 (0%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           K+YV+MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S    +
Sbjct: 7   KDYVNMYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFP- 65

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           DLVLTNALIVDYT                          +QDGVD +L +G  T+ + AEGL
Sbjct: 66  DLVLTNALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLIIGTNTDIIGAEGL 125

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGGIDTHIHFISP QI  A  SGVTTMIGGG GP++GTNATT T G  ++ SML+A
Sbjct: 126 IVTAGGIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMLKAT 185

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           + Y MN GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVA
Sbjct: 186 QNYPMNFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMDIQVA 245

Query: 241 IHT 243
           IHT
Sbjct: 246 IHT 248
```

tr Q8GLB3 **UreB (Fragment) [Helicobacter heilmannii]** 184 AA
Q8GLB3_HELHE align

```
 Score =  283 bits (725), Expect = 2e-75
 Identities = 142/180 (78%), Positives = 150/180 (82%)

Query: 21  DLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXXXXXXX 80
           DLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+ELDLV+TNALIVDYT
Sbjct: 3   DLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHELDLVITNALIVDYTGIYKADI 62

Query: 81  XXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTHIHFISPQQIP 140
                          +QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFISPQQIP
Sbjct: 63  GIKNGKIHGIGKAGNKDLQDGVCNRLCVGPATEALAAEGLIVTAGGIDTHIHFISPQQIP 122

Query: 141 TAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVSYEPS 200
           TAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+EEYAMNLG+L KGNVS+EP+
Sbjct: 123 TAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGKGNVSFEPA 182
```

tr Q8XXT1 **PROBABLE UREASE (ALPHA SUBUNIT) PROTEIN (EC 3.5.1.5)** 572
Q8XXT1_RALSO **[ureC]** AA
[Ralstonia solanacearum (Pseudomonas solanacearum)] align

```
 Score =  282 bits (722), Expect = 4e-75
 Identities = 145/243 (59%), Positives = 169/243 (68%), Gaps = 2/243 (0%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           + Y  M+GPTTGDRVRL DTDLI+EVE D T YGEE+KFGGGK IRDGM Q+   S
Sbjct: 8   RAYAEMFGPTTGDRVRLADTDLIVEVERDYTIYGEEVKFGGGKVIRDGMGQSQRESKDCA 67

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNALI+D+                          +Q GV   + +GP TE +A EG+
Sbjct: 68  DTVITNALIIDHWGIVKADIGLKHGRIAAIGKAGNPDIQPGV--TIVIGPGTEIIAGEGM 125

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           IVTAGG+DTHIHFI PQQI  A  SGVTTMIGGGTGPA GT ATT TPG  +L+ ML+AA
Sbjct: 126 IVTAGGVDTHIHFICPQQIDEALNSGVTTMIGGGTGPATGTYATTCTPGPWHLQRMLQAA 185

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           + Y MN+GFL KGN S    +LR+QI+AGAIG K+HEDWGSTPAAI  CL VAD+ D QVA
Sbjct: 186 DAYPMNIGFLGKGNGSLPGALREQIDAGAIGLKLHEDWGSTPAAIDCCLGVADDTDTQVA 245

Query: 241 IHT 243
           IHT
Sbjct: 246 IHT 248
```

tr Q8GLB6 **UreB (Fragment) [Helicobacter heilmannii]** 180 AA
Q8GLB6_HELHE align

```
 Score =  282 bits (721), Expect = 5e-75
 Identities = 141/180 (78%), Positives = 150/180 (83%)

Query: 21  DLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXXXXXXX 80
           DLILEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+ELDLV+TNALIVDYT
Sbjct: 1   DLILEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHELDLVITNALIVDYTGIYKADI 60

Query: 81  XXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTHIHFISPQQIP 140
                          +QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFISPQQIP
Sbjct: 61  GIKNGKIHGIGKAGNKDLQDGVCNRLCVGPATEALAAEGLIVTAGGIDTHIHFISPQQIP 120

Query: 141 TAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVSYEPS 200
           TAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+EEYAMNLG+L +GNVS+EP+
Sbjct: 121 TAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGQGNVSFEPA 180
```

tr Q8GLB2 **UreB (Fragment) [Helicobacter heilmannii]** 178 AA
Q8GLB2_HELHE align

```
 Score =  281 bits (720), Expect = 6e-75
 Identities = 141/178 (79%), Positives = 148/178 (82%)

Query: 16  RLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTXX 75
           RLGDTDL+LEVEHDCTTYGEEIKFGGGKTIRDGM QTNSPSS+ELDLV+TNALIVDYT
Sbjct: 1   RLGDTDLVLEVEHDCTTYGEEIKFGGGKTIRDGMGQTNSPSSHELDLVITNALIVDYTGI 60

Query: 76  XXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTHIHFIS 135
                                 +QDGV N LCVGPATEALAAEGLIVTAGGIDTHIHFIS
```

```
Sbjct: 61  YKADIGIKNGKIHGIGKAGNKDLQDGVCNRLCVGPATEALAAEGLIVTAGGIDTHIHFIS 120

Query: 136 PQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKG 193
           PQQIPTAFASG+TTMIGGGTGPADGTNATTITPGR NLK MLRA+EEYAMNLG+L KG
Sbjct: 121 PQQIPTAFASGITTMIGGGTGPADGTNATTITPGRWNLKEMLRASEEYAMNLGYLGKG 178
```

tr Q8YQZ0 Q8YQZ0_ANASP **Urease alpha subunit [alr3670] [Anabaena sp. (strain PCC 7120)]** 568 AA align

```
 Score =  281 bits (718), Expect = 1e-74
 Identities = 144/242 (59%), Positives = 170/242 (69%), Gaps = 3/242 (1%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYELD 61
           Y   YGPT GDR+RL DT+L ++VE D TTYG+E+KFGGGK IRDGM Q+  + +   +D
Sbjct: 10  YAETYGPTVGDRIRLADTELFIQVEQDFTTYGDEVKFGGGKVIRDGMGQSPIANADGAVD 69

Query: 62  LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLI 121
           LV+TNALI+D+                        +QD VD  + +GP TEALA EG+I
Sbjct: 70  LVITNALILDWWGIVKADIGIKDGKIFKIGKAGNPYIQDHVD--IIIGPGTEALAGEGMI 127

Query: 122 VTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 181
           +TAGGIDTHIHFI PQQI  A ASG+TTMIGGGTGPA GTNATT TPG  N+  ML+AA+
Sbjct: 128 LTAGGIDTHIHFICPQQIEVAIASGITTMIGGGTGPATGTNATTCTPGPWNMYRMLQAAD 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
            + MNLGFL KGN S    L +QI AGAIG K+HEDWG+TPA I  CL VADEYDVQVAI
Sbjct: 188 AFPMNLGFLGKGNASQPQGLVEQIFAGAIGLKLHEDWGTTPATIDTCLTVADEYDVQVAI 247

Query: 242 HT 243
           HT
Sbjct: 248 HT 249
```

tr Q6I6H5 Q6I6H5_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  280 bits (716), Expect = 2e-74
 Identities = 144/238 (60%), Positives = 169/238 (70%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                        +QDGVD++L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKASIGIKNGYIVGIGKAGNPDIQDGVDSSLIIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIGGG GP++GTNATT T G  ++ SML+A + Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMLKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
```

```
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQVAIHT 237
```

tr Q6I6I9 Q6I6I9_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  278 bits (712), Expect = 5e-74
 Identities = 144/238 (60%), Positives = 168/238 (70%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                        +QDGVD +L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLVIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIGGG GP++GTNATT T G  ++ SML+A + Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMLKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQVAIHT 237
```

tr Q6I6H3 Q6I6H3_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  278 bits (712), Expect = 5e-74
 Identities = 144/238 (60%), Positives = 168/238 (70%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                        +QDGVD +L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLIIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIGGG GP++GTNATT T G  ++ SML+A + Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMLKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMDIQVAIHT 237
```

tr Q6I6I3 **Urease beta subunit (Fragment) [ureB] [Campylobacter** 454

Q6I6I3_CAMLA **lari]** AA align

Score = 278 bits (711), Expect = 7e-74
Identities = 144/238 (60%), Positives = 167/238 (69%), Gaps = 1/238 (0%)

```
Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   + LDLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDF-LDLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                        +QDGVD +L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLVIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIGGG GP++GTNATT T G  ++ SML+A   Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMLKATHNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   L +ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQITAGACGLKIHEDWGATSSVIDASLKIADEMDIQVAIHT 237
```

tr Q7V3V2 **Urease alpha subunit (EC 3.5.1.5) [ureC]** 574
Q7V3V2_PROMM **[Prochlorococcus marinus (strain MIT 9313)]** AA align

Score = 277 bits (709), Expect = 1e-73
Identities = 142/242 (58%), Positives = 169/242 (69%), Gaps = 3/242 (1%)

```
Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYELD 61
           Y   YGPTTGDR+RL DT+LILEVE D TTYGEE+KFGGGK IRDGM Q+  S ++  +D
Sbjct: 10  YAETYGPTTGDRIRLADTELILEVERDFTTYGEEVKFGGGKVIRDGMGQSQQSRANGAVD 69

Query: 62  LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLI 121
            V+TNALI+D+                        + DG+D  + +GP TEA+A EG I
Sbjct: 70  TVITNALILDWWGIVKADIGLRDGRIVAIGKAGNPDITDGID--IVIGPGTEAIAGEGHI 127

Query: 122 VTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 181
           VTAG ID+HIHFI PQQI TA ASGVTTM+GGGTGPA GTNATT TPG  ++  ML+AAE
Sbjct: 128 VTAGAIDSHIHFICPQQIETALASGVTTMLGGGTGPATGTNATTCTPGAFHISRMLQAAE 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
              MNLGF  KGN S   +L +Q+ AGA G K+HEDWG+TPAAI  CL+VAD +DVQV I
Sbjct: 188 GLPMNLGFFGKGNASTAEALEEQVLAGACGLKLHEDWGTTPAAIDCCLSVADRFDVQVCI 247

Query: 242 HT 243
           HT
Sbjct: 248 HT 249
```

tr Q6I6J1 **Urease beta subunit (Fragment) [ureB] [Campylobacter** 454
Q6I6J1_CAMLA **lari]** AA align

```
 Score =  277 bits (709), Expect = 1e-73
 Identities = 144/238 (60%), Positives = 167/238 (69%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                        +QDGVD +L +G  T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLIIGTNTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIGGG GP++GTNATT T G  ++ SML+A + Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMLKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMDIQVAIHT 237
```

tr Q6I6I5 Q6I6I5_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  277 bits (708), Expect = 2e-73
 Identities = 143/238 (60%), Positives = 167/238 (70%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                        +QDGVD +L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLIIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIGGG GP++GTNATT T G  ++ SML+  + Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMLKTTQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMDIQVAIHT 237
```

tr Q6I6H1 Q6I6H1_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  276 bits (707), Expect = 2e-73
 Identities = 142/238 (59%), Positives = 167/238 (69%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGDFP-DLVLT 59
```

```
Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                          +QDGVD +L +G +T+ + AEGLI+TAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLVIGTSTDIIGAEGLIITAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIGGG GP++GTNATT T G  ++ SML+  + Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGGGIGPSEGTNATTCTSGAYHIHSMLKTTQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDASLNIADEMDIQVAIHT 237
```

tr Q5E728 Q5E728_VIBF1 **Urease alpha subunit (EC 3.5.1.5) [VF0673] [Vibrio fischeri (strain ATCC 700601 / ES114)]** 567 AA align

```
 Score =  276 bits (707), Expect = 2e-73
 Identities = 138/241 (57%), Positives = 169/241 (69%), Gaps = 2/241 (0%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDL 62
           Y +M+GPTTGDR+RL DT+L LEVE D TTYGEE+KFGGGK IRDGM Q+   +S  +D+
Sbjct: 9   YANMFGPTTGDRLRLADTELFLEVEKDFTTYGEEVKFGGGKVIRDGMGQSQVVNSECVDV 68

Query: 63  VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIV 122
           V+TNALI+D+                          +Q  VD  + VGPATE +A EG I+
Sbjct: 69  VITNALILDHWGIVKADIGIKDGRIFGIGKAGNPDVQPNVD--IVVGPATEVVAGEGKII 126

Query: 123 TAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
           TAGG+DTHIHFI PQQ      SGVTT IGGGTGP  GTNATT+TPG  N+  ML A ++
Sbjct: 127 TAGGVDTHIHFICPQQAEEGLTSGVTTFIGGGTGPVAGTNATTVTPGIWNMHRMLEAVDD 186

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
             +N+G   KG VS   +LR+QIEAGA+G KIHEDWG+TPAAIH+CLNVADE D+Q+AIH
Sbjct: 187 LPINVGLFGKGCVSKPEALREQIEAGAMGLKIHEDWGATPAAIHNCLNVADEMDIQIAIH 246

Query: 243 T 243
           +
Sbjct: 247 S 247
```

tr Q8DMV6 Q8DMV6_SYNEL **Urease alpha subunit [ureC] [Synechococcus elongatus (Thermosynechococcus elongatus)]** 572 AA align

```
 Score =  276 bits (705), Expect = 4e-73
 Identities = 136/242 (56%), Positives = 172/242 (70%), Gaps = 3/242 (1%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSY-ELD 61
           Y   YGPT GDR+RL DTDLI+E+EHD T YG+E+KFGGGK IRDGM Q+   ++   +D
Sbjct: 10  YAETYGPTVGDRLRLADTDLIIEIEHDYTHYGDEVKFGGGKVIRDGMGQSPIANAEGAVD 69

Query: 62  LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLI 121
           +V+TNA+I+D+                          Q+GVD  + +GP TEA+A EG+I
Sbjct: 70  VVITNAVILDWWGVVKADVGIKDGKIYKIGKAGNPYTQEGVD--IIIGPGTEAIAGEGMI 127
```

```
Query: 122 VTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 181
           +TAGGID HIHFI PQQI TA A+G+TTMIGGGTGPA GTNATT TPG  N+  ML+AA+
Sbjct: 128 LTAGGIDAHIHFICPQQIATAIAAGITTMIGGGTGPATGTNATTCTPGPWNIYRMLQAAD 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
            + +NLGFL KGN S     L +Q++AG +G K+HEDWGSTP AI  CL+VA++YD+QVAI
Sbjct: 188 AFPVNLGFLGKGNSSQPQGLIEQVQAGVVGLKLHEDWGSTPNAIDTCLSVAEDYDIQVAI 247

Query: 242 HT 243
           HT
Sbjct: 248 HT 249
```

tr Q4ZN06 Q4ZN06_PSESY **Urease (EC 3.5.1.5) [Psyr_4436] [Pseudomonas syringae pv. syringae B728a]** 566 AA align

```
 Score =  275 bits (702), Expect = 8e-73
 Identities = 137/241 (56%), Positives = 170/241 (69%), Gaps = 2/241 (0%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDL 62
           Y  M+GPT GD+VRL DT+L +EVE D TTYGEE+KFGGGK IRDGM Q     ++  +D
Sbjct: 8   YADMFGPTVGDKVRLADTELWIEVEKDFTTYGEEVKFGGGKVIRDGMGQGQLLAAEVVDT 67

Query: 63  VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIV 122
           ++TNALI+D+                          +Q  V   + VG ATE +A EG+I+
Sbjct: 68  LITNALIIDHWGIVKADVGIKNGRIAAIGKAGNPDIQPDV--TIAVGAATEVIAGEGMIL 125

Query: 123 TAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
           TAGG+DTHIHFI PQQI  A SGVTTMIGGGTGPA GTNATT+TPG  ++  ML+A++
Sbjct: 126 TAGGVDTHIHFICPQQIEEALMSGVTTMIGGGTGPATGTNATTVTPGPWHMARMLQASDS 185

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
           + MN+GF  KGNVS     L +Q++AGAIG K+HEDWG+TPAAI +CL+VADEYDVQVAIH
Sbjct: 186 FPMNIGFTGKGNVSLPGPLIEQVKAGAIGLKLHEDWGTTPAAIDNCLSVADEYDVQVAIH 245

Query: 243 T 243
           T
Sbjct: 246 T 246
```

tr Q9RYJ4 Q9RYJ4_DEIRA **Urease, alpha subunit [DRA0318] [Deinococcus radiodurans]** 568 AA align

```
 Score =  274 bits (701), Expect = 1e-72
 Identities = 135/246 (54%), Positives = 171/246 (68%), Gaps = 3/246 (1%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           ++Y  +YGPT GDRVRLGDT+L++EVE D TTYGEE+KFGGGK IRDG+ Q+++ +  +
Sbjct: 6   QQYADLYGPTVGDRVRLGDTELLIEVERDLTTYGEEVKFGGGKVIRDGLGQSSAATRDDA 65

Query: 61  ---DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAA 117
              DLV+TNALI+DY                        QDGV   L +  +TE +A
```

```
Sbjct: 66  NVPDLVITNALILDYWGVIKADVGVKNGRISAIGKAGNPGTQDGVTPGLTIAASTEIVAG 125

Query: 118 EGLIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSML 177
           EGL++TAGG+DTHIHFI+PQQ  TA  SGVTTMIGGGTGP  GT+ATT TPG+ ++  ML
Sbjct: 126 EGLVLTAGGVDTHIHFIAPQQCWTALESGVTTMIGGGTGPTAGTSATTCTPGQWHIHRML 185

Query: 178 RAAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDV 237
            +     +N G L KGN S +P L +QI AGA+G K+HEDWG+TPAAIH  L+VA++YDV
Sbjct: 186 ESLAGLPLNFGLLGKGNASTQPPLAEQIRAGALGLKLHEDWGTTPAAIHAALSVAEDYDV 245

Query: 238 QVAIHT 243
           QVAIHT
Sbjct: 246 QVAIHT 251
```

tr Q87VP0 Q87VP0_PSESM **Urease, alpha subunit [ureC] [Pseudomonas syringae (pv. tomato)]** 566 AA align

```
 Score =  274 bits (701), Expect = 1e-72
 Identities = 137/241 (56%), Positives = 170/241 (69%), Gaps = 2/241 (0%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDL 62
           Y  M+GPT GD+VRL DT+L +EVE D TTYGEE+KFGGGK IRDGM Q    ++  +D
Sbjct: 8   YADMFGPTVGDKVRLADTELWIEVEKDFTTYGEEVKFGGGKVIRDGMGQGQLLAADVVDT 67

Query: 63  VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIV 122
           ++TNALI+D+                          +Q  V   + VG ATE +A EG+I+
Sbjct: 68  LITNALIIDHWGIVKADVGIKNGRIAAIGKAGNPDIQPDV--TIAVGAATEVIAGEGMIL 125

Query: 123 TAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
           TAGG+DTHIHFI PQQI  A  SGVTTMIGGGTGPA GTNATT+TPG  ++  ML+A++
Sbjct: 126 TAGGVDTHIHFICPQQIEEALMSGVTTMIGGGTGPATGTNATTVTPGPWHMARMLQASDS 185

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
           + MN+GF  KGNVS    L +Q++AGAIG K+HEDWG+TPAAI +CL+VADEYDVQVAIH
Sbjct: 186 FPMNIGFTGKGNVSLPGPLIEQVKAGAIGLKLHEDWGTTPAAIDNCLSVADEYDVQVAIH 245

Query: 243 T 243
           T
Sbjct: 246 T 246
```

sp P16122 URE1_PROVU **Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) [ureC] [Proteus vulgaris]** 567 AA align

```
 Score =  274 bits (700), Expect = 1e-72
 Identities = 135/241 (56%), Positives = 166/241 (68%), Gaps = 2/241 (0%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDL 62
           Y  M+GPTTGDR+RL DT+L LE+E D TTYGEE+KFGGGK IRDGM Q+   S+  +D+
Sbjct: 9   YADMFGPTTGDRLRLADTELFLEIEQDFTTYGEEVKFGGGKVIRDGMGQSQVVSAECVDV 68

Query: 63  VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIV 122
```

```
          ++TNA+I+D+                              +Q  VD  + +GP TE +A EG I+
Sbjct: 69  LITNAIIIDHWGIVKADIGIKDGRITGIGKAGNPDVQPNVD--IVIGPGTEVVAGEGKII 126

Query: 123 TAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
           TAGG+DTHIHFI PQQ      SGVTT IGGGTGP  GTNAPT+TPG  N+  ML A +E
Sbjct: 127 TAGGVDTHIHFICPQQAEEGLISGVTTFIGGGTGPVAGTNATTVTPGIWNMHRMLEAVDE 186

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
             +N+G   KG VS   ++R+QIEAGAIG KIHEDWG+TP AIH+CLNVADE DVQVAIH
Sbjct: 187 LPINVGLFGKGCVSQPEAIREQIEAGAIGLKIHEDWGATPMAIHNCLNVADEMDVQVAIH 246

Query: 243 T 243
           +
Sbjct: 247 S 247
```

tr Q733J6 Q733J6_BACC1 **Urease alpha subunit (EC 3.5.1.5) [ureC] [Bacillus cereus (strain ATCC 10987)]** 570 AA align

```
 Score =  273 bits (698), Expect = 2e-72
 Identities = 134/245 (54%), Positives = 171/245 (69%), Gaps = 4/245 (1%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYE- 59
           K+Y  +YGPTTGD +RL DT L   +E + T YG+E  FGGGK+IRDGM Q +  +  +
Sbjct: 8   KQYADLYGPTTGDSIRLADTQLFAHIERNATVYGDEAVFGGGKSIRDGMGQNSQLTREQG 67

Query: 60  -LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAE 118
            +D+V+TNA+I+DYT                        + D +D  + +G +TE ++ E
Sbjct: 68  VVDVVITNAIIIDYTGIYKADIGIKDGKISAIGKSGNPSVMDNID--IIIGTSTEVISGE 125

Query: 119 GLIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLR 178
             IVTAGGIDTH+HFISPQQI TA ASG+TT+IGGGTGPA+GT ATTITPG  NL+ ML
Sbjct: 126 RKIVTAGGIDTHVHFISPQQIDTALASGITTLIGGGTGPAEGTKATTITPGSWNLRKMLE 185

Query: 179 AAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQ 238
           AAE + +NLGFL KGN S  P+L +QI AGAIG KIHEDWG+T +AI+H L +AD+YD+Q
Sbjct: 186 AAEAFPINLGFLGKGNSSSLPALEEQIFAGAIGLKIHEDWGATSSAINHSLQIADKYDIQ 245

Query: 239 VAIHT 243
           VAIHT
Sbjct: 246 VAIHT 250
```

sp P17086 URE1_PROMI **Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) [ureC] [Proteus mirabilis]** 567 AA align

```
 Score =  273 bits (697), Expect = 3e-72
 Identities = 137/241 (56%), Positives = 165/241 (67%), Gaps = 2/241 (0%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDL 62
           Y  M+GPTTGDR+RL DT+L LE+E D TTYGEE+KFGGGK IRDGM Q+   S+  +D+
Sbjct: 9   YADMFGPTTGDRLRLADTELFLEIEKDFTTYGEEVKFGGGKVIRDGMGQSQVVSAECVDV 68
```

```
Query: 63  VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIV 122
           ++TNA+I+DY                          +Q  VD  + +GP TE +A EG IV
Sbjct: 69  LITNAIILDYWGIVKADIGIKDGRIVGIGKAGNPDVQPNVD--IVIGPGTEVVAGEGKIV 126

Query: 123 TAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
           TAGGIDTHIHFI PQQ      SGVTT IGGGTGP  GTNATT+TPG  N+  ML A +E
Sbjct: 127 TAGGIDTHIHFICPQQAQEGLVSGVTTFIGGGTGPVAGTNATTVTPGIWNMYRMLEAVDE 186

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
             +N+G   EG VS   ++R+QI AGAIG KIHEDWG+TP AIH+CLNVADE DVQVAIH
Sbjct: 187 LPINVGLFGKGCVSQPEAIREQITAGAIGLKIHEDWGATPMAIHNCLNVADEMDVQVAIH 246

Query: 243 T 243
           +
Sbjct: 247 S 247
```

sp P73061 URE1_SYNY3 **Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase) [ureC] [Synechocystis sp. (strain PCC 6803)]** 569 AA align

```
 Score =  272 bits (696), Expect = 4e-72
 Identities = 136/242 (56%), Positives = 173/242 (71%), Gaps = 3/242 (1%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYELD 61
           Y   +GPT GD+VRL DT+I +EVE D  TYG+E+KFGGGK IRDGM Q+  S +   +D
Sbjct: 10  YAHTFGPTVGDKVRLADTELFIEVEQDYATYGDEVKFGGGKVIRDGMGQSPLSRAEGAVD 69

Query: 62  LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLI 121
           +V+TNALI+D+                          +QD V  ++ +GP+TEA+A EG+I
Sbjct: 70  VVITNALILDWWGIVKADVGIKNGRIYAIGKAGNPHIQDNV--SIIIGPSTEAIAGEGMI 127

Query: 122 VTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 181
           +TAGGID H+HFI PQQI TA ASGVTT++GGGTGPA GT ATT TPG  N+  ML+AA+
Sbjct: 128 LTAGGIDAHVHFICPQQIETALASGVTTLVGGGTGPAAGTKATTCTPGAWNIHRMLQAAD 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
            + +NLGFL KGN S   +L +QI+AGAIG K+HEDWG+TPAAI +CL VA++YDVQVAI
Sbjct: 188 GFPINLGFLGKGNGSQPAALAEQIKAGAIGLKLHEDWGTTPAAIDNCLGVAEDYDVQVAI 247

Query: 242 HT 243
           HT
Sbjct: 248 HT 249
```

tr Q7U3I3 Q7U3I3_SYNPX **Urease alpha subunit (EC 3.5.1.5) [ureC] [Synechococcus sp. (strain WH8102)]** 569 AA align

```
 Score =  272 bits (696), Expect = 4e-72
 Identities = 141/242 (58%), Positives = 165/242 (67%), Gaps = 3/242 (1%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSY-ELD 61
           Y   YGPTTGDRVRL DTDLILEVE D T YG+E+KFGGGK IRDGM Q+ +P +   +D
Sbjct: 10  YAETYGPTTGDRVRLADTDLILEVEKDYTVYGDEVKFGGGKVIRDGMGQSQTPRTEGAVD 69
```

```
Query: 62  LVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLI 121
           V+TNALI+D+                                Q GV   + VGP TEA+A EG I
Sbjct: 70  TVITNALILDWWGIVKADVGLKDGRIVGIGKAGNPDTQQGV--TIVVGPGTEAIAGEGHI 127

Query: 122 VTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAE 181
           +TAGGIDTHIHFI PQQI TA ASGVTT++GGGTGPA GTNATT TPG  ++  ML+AAE
Sbjct: 128 LTAGGIDTHIHFICPQQIETALASGVTTLMGGGTGPATGTNATTCTPGAFHIGRMLQAAE 187

Query: 182 EYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAI 241
              +NLGF  KGN S   +L +Q+ AGA G K+HEDWG+TPA I  CL+VAD  DVQV I
Sbjct: 188 GLPVNLGFFGKGNASTPEALEEQVRAGACGLKLHEDWGTTPATIDACLSVADRMDVQVCI 247

Query: 242 HT 243
           HT
Sbjct: 248 HT 249
```

tr O52305 O52305_SYNP2 **Urease alpha subunit [ureC] [Synechococcus sp. (strain PCC 7002) (Agmenellum quadruplicatum)]** 569 AA align

```
 Score =  272 bits (696), Expect = 4e-72
 Identities = 137/244 (56%), Positives = 169/244 (69%), Gaps = 3/244 (1%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSP-SSYE 59
           + Y   YGPT GDR+RL DT+L LEVE D TTYG+E+KFGGGK IRDGM Q+  P +
Sbjct: 8   RAYAETYGPTVGDRLRLADTELWLEVEQDFTTYGDEVKFGGGKVIRDGMGQSAVPRADGA 67

Query: 60  LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEG 119
           +D V+TNALIVD+                                +QD +D  + +GP TEA+A EG
Sbjct: 68  VDTVITNALIVDWWGIVKADVGIKDGKIYKIGKAGNPDIQDNID--IIIGPGTEAIAGEG 125

Query: 120 LIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRA 179
            I+TAGGID+H+HFI PQQI  A ASG+TTMIGGGTGPA GTNATT TPG  ++  ML A
Sbjct: 126 HILTAGGIDSHVHFICPQQIEVAIASGITTMIGGGTGPATGTNATTCTPGEWHISRMLEA 185

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           AE + +NLGF  KGN +    L +Q+ AG IG K+HEDWG+TPAAI +CL+VAD++DVQV
Sbjct: 186 AEGFPINLGFTGKGNSAKPEGLIEQVRAGVIGLKLHEDWGTTPAAIDNCLSVADQFDVQV 245

Query: 240 AIHT 243
           AIHT
Sbjct: 246 AIHT 249
```

tr Q6I6H7 Q6I6H7_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  272 bits (695), Expect = 5e-72
 Identities = 141/238 (59%), Positives = 166/238 (69%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
```

```
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                          +QDGVD +L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLVIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIG   GP++GTNATT T G  ++ SML++ + Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGARIGPSEGTNATTCTSGAYHIHSMLKSTQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVAIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQVAIHT 237
```

tr Q6I6I1 Q6I6I1_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  271 bits (692), Expect = 1e-71
 Identities = 140/238 (58%), Positives = 165/238 (68%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                          +QDGVD +L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLVIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A  SGVTTMIG   GP++GTNATT T G  ++ SML++   Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGAPIGPSEGTNATTCTSGAYHIHSMLKSTHNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+Q+AIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQIAIHT 237
```

tr Q6I6H9 Q6I6H9_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  271 bits (692), Expect = 1e-71
 Identities = 140/238 (58%), Positives = 165/238 (68%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                          +QDGVD +L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVDPSLVIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
```

```
           GIDTHIHFISP QI  A  SGVTTMIG   GP++GTNATT T G  ++ SML++   Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGAPIGPSEGTNATTCTSGAYHIHSMLKSTHNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+Q+AIHT
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQIAIHT 237
```

tr Q6FD83 Q6FD83_ACIAD **Urease alpha subunit (EC 3.5.1.5) [ureC] [Acinetobacter sp. (strain ADP1)]** 566 AA align

```
 Score =  270 bits (691), Expect = 1e-71
 Identities = 135/243 (55%), Positives = 170/243 (69%), Gaps = 2/243 (0%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYEL 60
           + Y  M+GPT GDRVRL DT+L +EVE D TTYGEE+KFGGGK IRDGM Q+   +
Sbjct: 6   RAYAEMFGPTVGDRVRLADTELFIEVEQDLTTYGEEVKFGGGKVIRDGMGQSQLLADEVA 65

Query: 61  DLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGL 120
           D V+TNALIVD+                          +Q   D  + +G ATE +A EG
Sbjct: 66  DTVITNALIVDWWGIVKADVGLKNGRIWKIGKAGNPDIQP--DITIPLGAATEVIAGEGQ 123

Query: 121 IVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAA 180
           I+TAGGIDTHIH+I PQQ+ TA  SGVTTM+GGGTGPA GT+ATT+TPG  ++ +ML+A
Sbjct: 124 ILTAGGIDTHIHWICPQQVETALMSGVTTMVGGGTGPAAGTSATTVTPGPWHIGTMLQAI 183

Query: 181 EEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVA 240
           ++  MN+G L KGN+S     +R+QI+AG +G K+HEDWGSTPAAI +CL+VADE+DVQVA
Sbjct: 184 DDLPMNIGLLGKGNLSLPDPIREQIKAGVVGLKLHEDWGSTPAAIDNCLSVADEFDVQVA 243

Query: 241 IHT 243
           IHT
Sbjct: 244 IHT 246
```

tr Q9HUU5 Q9HUU5_PSEAE **Urease alpha subunit [ureC] [Pseudomonas aeruginosa]** 566 AA align

```
 Score =  270 bits (689), Expect = 3e-71
 Identities = 136/241 (56%), Positives = 165/241 (68%), Gaps = 2/241 (0%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDL 62
           Y  M+GPT GDRVRL DTDL +EVE D T YGEE+KFGGGK IRDGM Q+   ++  +D
Sbjct: 8   YADMFGPTVGDRVRLADTDLWIEVERDFTVYGEEVKFGGGKVIRDGMGQSQLGAAQVVDT 67

Query: 63  VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIV 122
           V+TNALI+D+                          +Q GV  N+ +G  TE +A EG+I+
Sbjct: 68  VITNALILDHWGVVKADVGLKDGRIQAIGKAGNPDIQPGV--NIAIGAGTEVIAGEGMIL 125

Query: 123 TAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
           TAGGIDTHIHFI PQQI  A  SGVTTMIGGGTGPA GTNATT T G  ++  ML+AA+
Sbjct: 126 TAGGIDTHIHFICPQQIEEALMSGVTTMIGGGTGPAAGTNATTCTSGPWHMARMLQAADA 185
```

```
Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
           + MN+GF  KGN S    L +Q+ AGAIG K+HEDWGSTPAAI +CL VA+ +D+QVAIH
Sbjct: 186 FPMNIGFTGKGNASLPLPLEEQVLAGAIGLKLHEDWGSTPAAIDNCLEVAERHDIQVAIH 245

Query: 243 T 243
           T
Sbjct: 246 T 246
```

tr Q88J04 Q88J04_PSEPK **Urease, alpha subunit [ureC] [Pseudomonas putida (strain KT2440)]** 567 AA align

```
 Score =  270 bits (689), Expect = 3e-71
 Identities = 140/241 (58%), Positives = 164/241 (67%), Gaps = 2/241 (0%)

Query: 3   YVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDL 62
           Y  M+GPT GDRVRL DT L +EVE D T YGEE+KFGGGK IRDGM Q    ++  +DL
Sbjct: 9   YADMFGPTVGDRVRLADTALWVEVEKDFTIYGEEVKFGGGKVIRDGMGQGQMLAAEAMDL 68

Query: 63  VLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIV 122
           VLTNALI+D+                          +Q GV  N+ VGP TE +AAEG IV
Sbjct: 69  VLTNALIIDHWGIVKADIGIKHGRIAVIGKAGNPDVQPGV--NVPVGPGTEVIAAEGKIV 126

Query: 123 TAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEE 182
           TAGG+D+HIHFI PQQ+  A  SGVTT IGGGTGPA GTNATT TPG  L  ML+AA+
Sbjct: 127 TAGGVDSHIHFICPQQVDEALNSGVTTFIGGGTGPATGTNATTCTPGPWYLARMLQAADS 186

Query: 183 YAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIH 242
             +N+G L KGN S   +LR+QI AGA+G K+HEDWGSTPAAI  CL VA+E D+QVAIH
Sbjct: 187 LPINIGLLGKGNASRPDALREQIAAGAVGLKLHEDWGSTPAAIDCCLGVAEEMDIQVAIH 246

Query: 243 T 243
           T
Sbjct: 247 T 247
```

tr Q7V1B6 Q7V1B6_PROMP **Urease alpha subunit (EC 3.5.1.5) [ureC] [Prochlorococcus marinus subsp. pastoris (strain CCMP 1378 / MED4)]** 569 AA align

```
 Score =  268 bits (686), Expect = 6e-71
 Identities = 136/244 (55%), Positives = 167/244 (67%), Gaps = 3/244 (1%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYE 59
           K Y   YGPT GDRVRL DT+LI+EVE D TTYG+E+KFGGGK IRDGM Q+  +
Sbjct: 8   KTYAQTYGPTKGDRVRLADTELIIEVEKDFTTYGDEVKFGGGKVIRDGMGQSQVTREDGA 67

Query: 60  LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEG 119
           +D V+TNALIVD+                          +QD +  N+ +G *TE +A EG
Sbjct: 68  VDTVITNALIVDWWGIVKADVGLKDGKIYEIGKAGNPDIQDNI--NIIIGSSTEVIAGEG 125

Query: 120 LIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRA 179
            I+TAG IDTHIHFI PQQI TA ASGVTTM+GGGTGPA GTNATT TPG  ++  M+++
Sbjct: 126 HILTAGSIDTHIHFICPQQIETALASGVTTMLGGGTGPATGTNATTCTPGAFHISRMIQS 185
```

```
Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           AE + +NLGF  KGN S E +L +Q+ AGA G K+HEDWG+TP+ I+ CLNVAD  DVQV
Sbjct: 186 AEAFPVNLGFFGKGNSSNETNLFEQVNAGACGLKLHEDWGTTPSTINSCLNVADTLDVQV 245

Query: 240 AIHT 243
           IHT
Sbjct: 246 CIHT 249
```

tr Q9L644 **UreC [ureC] [Prochlorococcus marinus]** 569 AA
Q9L644_PROMA align

```
 Score =  268 bits (686), Expect = 6e-71
 Identities = 136/244 (55%), Positives = 167/244 (67%), Gaps = 3/244 (1%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTN-SPSSYE 59
           K Y   YGPT GDRVRL DT+LI+EVE D TTYG+E+KFGGGK IRDGM Q+  + 
Sbjct: 8   KTYAQTYGPTKGDRVRLADTELIIEVEKDFTTYGDEVKFGGGKVIRDGMGQSQVTREDGA 67

Query: 60  LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEG 119
           +D V+TNALIVD+                          +QD +  N+ +G +TE +A EG
Sbjct: 68  VDTVITNALIVDWWGIVKADVGLKDGKIYEIGKAGNPDIQDNI--NIIIGSSTEVIAGEG 125

Query: 120 LIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRA 179
           I+TAG IDTHIHFI PQQI TA ASGVTTM+GGGTGPA GTNATT TPG  ++  M+++
Sbjct: 126 HILTAGSIDTHIHFICPQQIETALASGVTTMLGGGTGPATGTNATTCTPGAFHISRMIQS 185

Query: 180 AEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQV 239
           AE + +NLGF  KGN S E +L +Q+ AGA G K+HEDWG+TP+ I+ CLNVAD  DVQV
Sbjct: 186 AEAFPVNLGFFGKGNSSNETNLFEQVNAGACGLKLHEDWGTTPSTINSCLNVADTLDVQV 245

Query: 240 AIHT 243
           IHT
Sbjct: 246 CIHT 249
```

sp P77837 **Urease alpha subunit (EC 3.5.1.5) (Urea amidohydrolase)** 569
URE1_BACSU **[ureC]** AA
[Bacillus subtilis] align

```
 Score =  268 bits (685), Expect = 7e-71
 Identities = 131/245 (53%), Positives = 169/245 (68%), Gaps = 2/245 (0%)

Query: 1   KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYE- 59
           +EY  ++GPTTGD++RLGDTDL +EVE D T YGEE+ FGGGKTIRDGM Q    +  +
Sbjct: 6   EEYAELFGPTTGDKIRLGDTDLWIEVEKDFTVYGEEMIFGGGKTIRDGMGQNGRITGKDG 65

Query: 60  -LDLVLTNALIVDYTXXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAE 118
            LDLV+TN +++DYT                        + DGVD ++ +G  TE ++ E
Sbjct: 66  ALDLVITNVVLLDYTGIVKADVGVKDGRIVGVGKSGNPDIMDGVDPHMVIGAGTEVISGE 125

Query: 119 GLIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLR 178
           G I+TAGG+DTHIHFI PQQ+  A +SGVTT++GGGTGPA G+ ATT T G   +  ML
Sbjct: 126 GKILTAGGVDTHIHFICPQQMEVALSSGVTTLLGGGTGPATGSKATTCTSGAWYMARMLE 185
```

```
Query: 179 AAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQ 238
           AAEE+ +N+GFL KGN S +  L +Q+EAGAIG K+HEDWG+TP+AI  C+ V DE D+Q
Sbjct: 186 AAEEFPINVGFLGKGNASDKAPLIEQVEAGAIGLKLHEDWGTTPSAIKTCMEVVDEADIQ 245

Query: 239 VAIHT 243
           VAIHT
Sbjct: 246 VAIHT 250
```

tr Q6I6I8 Q6I6I8_CAMLA **Urease beta subunit (Fragment) [ureB] [Campylobacter lari]** 454 AA align

```
 Score =  268 bits (685), Expect = 7e-71
 Identities = 140/238 (58%), Positives = 165/238 (68%), Gaps = 1/238 (0%)

Query: 6   MYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTIRDGMSQTNSPSSYELDLVLT 65
           MYGPTT DRVRL DTDLIL VE D T YGEE+KFGGGK IRDGM+Q+ S   +  DLVLT
Sbjct: 1   MYGPTTNDRVRLADTDLILRVEKDYTLYGEEVKFGGGKNIRDGMAQSVSEGEFP-DLVLT 59

Query: 66  NALIVDYTXXXXXXXXXXXXXXXXXXXXXXXMQDGVDNNLCVGPATEALAAEGLIVTAG 125
           NALIVDYT                        +QDGV+ +L +G +T+ + AEGLIVTAG
Sbjct: 60  NALIVDYTGIYKADIGIKNGYIVGIGKAGNPDIQDGVNPSLVIGTSTDIIGAEGLIVTAG 119

Query: 126 GIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNATTITPGRANLKSMLRAAEEYAM 185
           GIDTHIHFISP QI  A SGVTTMIG  GP++GTNATT T G  ++ SML+A + Y M
Sbjct: 120 GIDTHIHFISPTQIECALYSGVTTMIGARIGPSEGTNATTCTSGAYHIHSMLKATQNYPM 179

Query: 186 NLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTPAAIHHCLNVADEYDVQVAIHT 243
           N GFL KGN S + +L++QI AGA G KIHEDWG+T + I   LN+ADE D+QVAI T
Sbjct: 180 NFGFLGKGNSSNKNALKEQIIAGACGLKIHEDWGATSSVIDTSLNIADEMDIQVAIQT 237
```

```
Database: EXPASY/UniProtKB
    Posted date:  Jun 6, 2005 10:37 AM
  Number of letters in database: 640,866,274
  Number of sequences in database:  1,974,938

Lambda     K      H
   0.315    0.135    0.393

Gapped
Lambda     K      H
   0.267   0.0410    0.140


Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
length of query: 243
length of database: 640,866,274
effective HSP length: 122
effective length of query: 121
effective length of database: 399,923,838
effective search space: 48390784398
effective search space used: 48390784398
```

```
T: 11
A: 40
X1: 16 ( 7.3 bits)
X2: 38 (14.6 bits)
X3: 64 (24.7 bits)
S1: 42 (22.0 bits)
S2: 72 (32.3 bits)

Wallclock time: 28 seconds
```

ExPASy Home page Site Map Search ExPASy Contact us Proteomics tools Swiss-Prot

UniProtKB/TrEMBL entry Q9RGP5

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**Q9RGP5_HELFE**
Primary accession number	**Q9RGP5**
Secondary accession numbers	None
Entered in TrEMBL in	Release 13, May 2000
Sequence was last modified in	Release 13, May 2000
Annotations were last modified in	Release 26, March 2004

Name and origin of the protein

Protein name	**Urease [Fragment]**
Synonyms	None
Gene name	**Name: ureB**
From	Helicobacter felis [TaxID: 214]
Taxonomy	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.

References

[1] NUCLEOTIDE SEQUENCE.
STRAIN=ATCC51211;
Weir S.C., Stock F., Gill V.J., Fischer S.H.;
Submitted (DEC-1998) to the EMBL/GenBank/DDBJ databases.

Comments

None

Cross-references

EMBL	AF116580; AAF21996.1; -; Genomic_DNA.	[EMBL / GenBank / DDBJ] [CoDingSequence]
HSSP	P14917; 1E9Y. [HSSP ENTRY / PDB]	
SMR	Q9RGP5; 1-243.	
GO	GO:0016787; Molecular function: hydrolase activity *(inferred from electronic annotation).*	
	GO:0016151; Molecular function: nickel ion binding *(inferred from electronic annotation).*	
	GO:0009039; Molecular function: urease activity *(inferred from electronic annotation).*	
	GO:0006807; Biological process: nitrogen compound metabolism *(inferred from electronic annotation).*	
	QuickGo	

	view.
InterPro	IPR006680; Amidohydro_1. IPR005848; Urease_alpha. IPR011612; Urease_alpha_N. Graphical view of domain structure.
Pfam	PF01979; Amidohydro_1; 1. PF00449; Urease_alpha; 1. Pfam graphical view of domain structure.
PROSITE	PS01120; UREASE_1; 1.
HOGENOM	[Family / Alignment / Tree]
ProtoMap	Q9RGP5.
PRESAGE	Q9RGP5.
ModBase	Q9RGP5.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

None

Features

Feature table viewer

Key	From	To	Length	Description
NON_TER	1	1		
NON_TER	243	243		

Sequence information

Length: **243 AA** [This is the length of the partial sequence] Molecular weight: **25607 Da** [This is the MW of the partial sequence] CRC64: **A9E2764C95B4BC62** [This is a checksum on the sequence]

```
        10          20          30          40          50          60
KEYVSMYGPT TGDRVRLGDT DLILEVEHDC TTYGEEIKFG GGKTIRDGMS QTNSPSSYEL

        70          80          90         100         110         120
DLVLTNALIV DYTGIYKADI GIKDGKIAGI GKAGNKDMQD GVDNNLCVGP ATEALAAEGL

       130         140         150         160         170         180
IVTAGGIDTH IHFISPQQIP TAFASGVTTM IGGGTGPADG TNATTITPGR ANLKSMLRAA

       190         200         210         220         230         240
EEYAMNLGFL AKGNVSYEPS LRDQIEAGAI GFKIHEDWGS TPAAIHHCLN VADEYDVQVA

IHT
```

Q9RGP5 in FASTA format

View entry in original UniProtKB/TrEMBL format
View entry in raw text format (no links)
Request for annotation of this UniProtKB/TrEMBL entry

EBI Dbfetch

```
ID   AF116580   standard; genomic DNA; PRO; 732 BP.
XX
AC   AF116580;
XX
SV   AF116580.1
XX
DT   02-JAN-2000 (Rel. 62, Created)
DT   15-APR-2005 (Rel. 83, Last updated, Version 2)
XX
DE   Helicobacter felis strain ATCC51211 urease (ureB) gene, partial cds.
XX
KW   .
XX
OS   Helicobacter felis
OC   Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales;
OC   Helicobacteraceae; Helicobacter.
XX
RN   [1]
RP   1-732
RA   Weir S.C., Stock F., Gill V.J., Fischer S.H.;
RT   "Helicobacter species differentiation based on DNA sequence variation in a
RT   region of the ureB gene";
RL   Unpublished.
XX
RN   [2]
RP   1-732
RA   Weir S.C., Stock F., Gill V.J., Fischer S.H.;
RT   ;
RL   Submitted (28-DEC-1998) to the EMBL/GenBank/DDBJ databases.
RL   CC/CPD, NIH, 10 Center Drive, Bethesda, MD 20892, USA
XX
FH   Key             Location/Qualifiers
FH
FT   source          1..732
FT                   /db_xref="taxon:214"
FT                   /mol_type="genomic DNA"
FT                   /organism="Helicobacter felis"
FT                   /strain="ATCC51211"
FT   CDS             <1..>732
FT                   /codon_start=3
FT                   /db_xref="GOA:Q9RGP5"
FT                   /db_xref="HSSP:1E9Y"
FT                   /db_xref="InterPro:IPR005848"
FT                   /db_xref="InterPro:IPR006680"
FT                   /db_xref="InterPro:IPR011612"
FT                   /db_xref="UniProt/TrEMBL:Q9RGP5"
FT                   /transl_table=11
FT                   /gene="ureB"
FT                   /product="urease"
FT                   /protein_id="AAF21996.1"
FT                   /translation="KEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIKFGGGKTI
FT                   RDGMSQTNSPSSYELDLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNKDMQDGVDN
FT                   NLCVGPATEALAAEGLIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGGTGPADGTNA
```

```
FT                   TTITPGRANLKSMLRAAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGFKIHEDWGSTP
FT                   AAIHHCLNVADEYDVQVAIHT"
XX
SQ   Sequence 732 BP; 199 A; 164 C; 190 G; 178 T; 1 other;
     gaaaagaata tgtttcnatg tatggtccca ctaccgggga tcgtgttaga ctcggcgata        60
     ctgatttgat cttagaagtg gaacatgatt gcaccactta tggcgaagag atcaaatttg       120
     ggggcggaaa aactatccgt gatgggatga gtcaaaccaa tagccctagc tcttatgagt       180
     tagatttggt gctcactaac gccctcattg tggactatac gggcatttac aaagccgaca       240
     ttgggattaa ggatggcaag attgcaggca ttggcaaagc tggcaataag gacatgcaag       300
     atggcgtaga taataatctg tgcgtaggtc ctgctacaga ggctttggca gctgaaggct       360
     tgattgtaac cgctggtggc atcgatacgc atattcactt tatttctccc caacaaatcc       420
     ctactgcttt tgccagcggg gttacaacca tgattggagg aggcacagga cctgcggatg       480
     gcacgaatgc gaccaccatc actcccggac gcgctaatct aaaaagtatg ttgcgtgcag       540
     ccgaagaata tgccatgaat ctaggctttt tggctaaggg gaatgtgtct tacgaaccct       600
     ctttacgcga tcagattgaa gcaggggcga ttggttttaa aatccacgaa gactggggaa       660
     gcacacctgc agccatccac cactgcctca atgtcgccga tgagtatgat gtgcaagtcg       720
     ccatccacac cg                                                          732
//
```


```
LOCUS       HFURE                   2619 bp    DNA     linear   BCT 18-APR-2005
DEFINITION  H.felis ureA and ureB genes.
ACCESSION   X69080
VERSION     X69080.1  GI:396160
KEYWORDS    ureA gene; urease; ureB gene.
SOURCE      Helicobacter felis
  ORGANISM  Helicobacter felis
            Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales;
            Helicobacteraceae; Helicobacter.
REFERENCE   1  (bases 1 to 2619)
  AUTHORS   Ferrero,R.L. and Labigne,A.
  TITLE     Cloning, expression and sequencing of Helicobacter felis urease
            genes
  JOURNAL   Mol. Microbiol. 9 (2), 323-333 (1993)
   PUBMED   8412683
REFERENCE   2  (bases 1 to 2619)
  AUTHORS   Ferrero,R.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-NOV-1992) R.L. Ferrero, Institut Pasteur, Unite des
            Enterobacteries, 28 rue du Docteur Roux, 75724 Paris Cedex 15,
            FRANCE
COMMENT     Related sequence: Labigne, A., J. Bacteriol.173:1920-1931 (1991).
FEATURES             Location/Qualifiers
     source          1..2619
                     /organism="Helicobacter felis"
                     /mol_type="genomic DNA"
                     /isolate="ATCC 49179"
                     /db_xref="taxon:214"
     gene            43..756
                     /gene="ureA"
     CDS             43..756
                     /gene="ureA"
                     /EC_number="3.5.1.5"
                     /codon_start=1
                     /transl_table=11
                     /product="urease alpha-6 subunit"
                     /protein_id="CAA48825.1"
                     /db_xref="GI:396161"
                     /db_xref="GOA:Q08715"
                     /db_xref="InterPro:IPR002019"
                     /db_xref="InterPro:IPR002026"
                     /db_xref="InterPro:IPR008223"
                     /db_xref="UniProt/Swiss-Prot:Q08715"
                     /translation="MKLTPKELDKLMLHYAGRLAEEALARGVKLNYTEAVALISGRVM
```

```
                     EKARDGNKSVADLMQEGRTWLKKENVMDGVASMIHEVGIEANFPDGTKLVTIHTPVED
                     NGKLAPGEVFLKNEDITINAGKEAISLKVKNKGDRPVQVGSHFHFFEVNKLLDFDRAK
                     SFCKRLDIASGTAVRFEPGEEKSVELIDIGGNKRIYGFNSLVDRQADADGKKLGLKRA
                     KEKGFGSVNCGCEATKDKQ"
     gene            766..2475
                     /gene="ureB"
     CDS             766..2475
                     /gene="ureB"
                     /EC_number="3.5.1.5"
                     /codon_start=1
                     /transl_table=11
                     /product="urease beta-6 subunit"
                     /protein_id="CAA48826.1"
                     /db_xref="GI:396162"
                     /db_xref="GOA:Q08716"
                     /db_xref="InterPro:IPR005848"
                     /db_xref="InterPro:IPR006680"
                     /db_xref="InterPro:IPR008295"
                     /db_xref="InterPro:IPR011059"
                     /db_xref="UniProt/Swiss-Prot:Q08716"
                     /translation="MKKISRKEYVSMYGPTTGDRVRLGDTDLILEVEHDCTTYGEEIK
                     FGGGKTIRDGMSQTNSPSSYELDLVLTNALIVDYTGIYKADIGIKDGKIAGIGKAGNK
                     DMQDGVDNNLCVGPATEALAAEGLIVTAGGIDTHIHFISPQQIPTAFASGVTTMIGGG
                     TGPADGTNATTITPGRANLKSMLRAAEEYAMNLGFLAKGNVSYEPSLRDQIEAGAIGF
                     KIHEDWGSTPAAIHHCLNVADEYDVQVAIHTDTLNEAGCVEDTLEAIAGRTIHTFHTE
                     GAGGGHAPDVIKMAGEFNILPASTNPTIPFTKNTEAEHMDMLMVCHHLDKSIKEDVQF
                     ADSRIRPQTIAAEDQLHDMGIFSITSSDSQAMGRVGEVITRTWQTADKNKKEFGRLKE
                     EKGDNDNFRIKRYISKYTINPGIAHGISDYVGSVEVGKYADLVLWSPAFFGIKPNMII
                     KGGFIALSQMGDANASIPTPQPVYYREMFGHHGKNKFDTNITFVSQAAYKAGIKEELG
                     LDRAAPPVKNCRNITKKDLKFNDVTAHIDVNPETYKVKVDGKEVTSKAADELSLAQLY
                     NLF"
ORIGIN
        1 tgatagcttg gctaccaata gaaattcaat aaggagttta ggatgaaact aacgcctaaa
       61 gaactagaca agttaatgct ccattatgcg ggcagattgg cagaagaagc gttggcgcgt
      121 ggtgtgaaac tcaattacac cgaagcggtc gcgctcatta gcgggcgtgt gatggaaaag
      181 gcgcgtgatg gtaataaaag cgtggcggat ttgatgcaag aaggcaggac ttggcttaaa
      241 aaagaaaatg tgatggacgg cgtagcaagc atgattcatg aagtggggat tgaagctaac
      301 ttccccgatg gaaccaagct tgtaactatc cacactccgg tagaggataa tggcaaatta
      361 gccccccggcg aggtcttctt aaaaaatgag gacattacta ttaacgccgg caaagaagcc
      421 attagcttga aagtgaaaaa taaaggcgat cgtcctgtgc aggtgggatc acatttccac
      481 ttcttcgaag tgaataagct cttggacttc gatcgcgcaa aaagcttttg caaacgccta
      541 gacattgcat ctggaacagc ggtgcgcttt gaacccgggg aggaaaaaag tgtggaactc
      601 attgacatcg gcgggaataa gcgcatctat ggctttaatt ctttggtgga tcgccaagcc
      661 gatgccgatg gtaaaaaact cggcttaaaa cgcgctaaag aaaaaggttt tgggtctgta
      721 aactgcggtt gtgaagcgac taaagataaa caataaggaa aaaccatgaa aaagatttca
      781 cgaaaagaat atgtttctat gtatggtccc actaccgggg atcgtgttag actcggcgac
      841 actgatttga tcttagaagt ggagcatgat tgcaccactt atggtgaaga gatcaaattt
      901 gggggcggta aaactatccg tgatgggatg agtcaaacca atagccctag ctcttatgaa
      961 ttagatttgg tgctcactaa cgccctcatt gtggactata cgggcattta caaagccgac
     1021 attgggatta aagacggcaa gattgcaggc attggcaagg caggcaataa ggacatgcaa
     1081 gatggcgtag ataataatct ttgcgtaggt cctgctacag aggctttggc agctgagggc
     1141 ttgattgtaa ccgctggtgg catcgatacg catattcact ttatctctcc ccaacaaatc
     1201 cctactgctt ttgccagcgg ggttacaacc atgattggag gaggcacagg acctgcggat
     1261 ggcacgaatg cgaccaccat cactcccgga cgcgctaatc taaaaagtat gttgcgtgca
     1321 gccgaagaat acgccatgaa tctaggcttt ttggctaagg ggaatgtgtc ttacgaaccc
     1381 tctttacgcg atcagattga agcaggggcg attggtttta aaatccacga agactgggga
     1441 agcacacctg cagctattca ccactgcctc aatgtcgccg atgaatacga tgtgcaagtg
     1501 gctatccaca ccgataccct taacgaggcg ggctgtgtag aagacaccct agaggcgatt
     1561 gccgggcgca ccatccatac cttccacact gaaggggctg ggggtggaca cgctccagat
     1621 gttatcaaaa tggcagggga atttaacatt ctacccgcct ctactaaccc gaccattcct
```

```
     1681 ttcaccaaaa acactgaagc cgagcacatg gacatgttaa tggtgtgcca ccacttggat
     1741 aaaagtatca aggaagatgt gcagtttgcc gattcgagga ttcgccccca aactatcgcg
     1801 gctgaagacc aactccatga catggggatc ttttctatca ccagctccga ctctcaggct
     1861 atgggacgcg taggcgaggt gatcacacgc acttggcaga cagcagacaa aaacaaaaaa
     1921 gagtttgggc gcttgaaaga ggaaaaaggc gataacgaca acttccgcat caaacgctac
     1981 atctctaaat acaccatcaa ccccgggatc gcgcatggga tttctgacta tgtgggctct
     2041 gtggaagtgg gcaaatacgc cgacctcgtg ctttggagtc cggctttctt tggcattaag
     2101 cccaatatga ttattaaggg cggatttatt gcgctctctc aaatgggcga tgccaatgcg
     2161 tctattccca cccctcagcc cgtctattac cgtgaaatgt ttggacacca tgggaaaaac
     2221 aaattcgaca ccaatatcac tttcgtgtcc caagcggctt acaaggcagg gatcaaagaa
     2281 gaactagggc tagatcgcgc ggcaccgcca gtgaaaaact gtcgcaatat cactaaaaag
     2341 gacctcaaat tcaacgatgt gaccgcacat attgatgtca accctgaaac ctataaggtg
     2401 aaagtggatg gcaaagaggt aacctctaaa gcagcagatg aattgagcct agcgcaactt
     2461 tataatttgt tctaggaggc taaggagggg gatagagggg gttaatttag aggggagtca
     2521 ttgatttacc tttgctagtt tataatggat ttaagagagg tttttttcg tgttttatac
     2581 cgcgttgaaa ccctcaaatc tttaccaaaa ggatggtaa
//
```

	view.
InterPro	IPR006680; Amidohydro_1. IPR005848; Urease_alpha. IPR011612; Urease_alpha_N. Graphical view of domain structure.
Pfam	PF01979; Amidohydro_1; 1. PF00449; Urease_alpha; 1. Pfam graphical view of domain structure.
PROSITE	PS01120; UREASE_1; 1.
HOGENOM	[Family / Alignment / Tree]
ProtoMap	Q9RGP5.
PRESAGE	Q9RGP5.
ModBase	Q9RGP5.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

None

Features

Feature table viewer

Key	From	To	Length	Description
NON_TER	1	1		
NON_TER	243	243		

Sequence information

Length: **243 AA** [This is the length of the partial sequence] Molecular weight: **25607 Da** [This is the MW of the partial sequence] CRC64: **A9E2764C95B4BC62** [This is a checksum on the sequence]

```
        10         20         30         40         50         60
KEYVSMYGPT TGDRVRLGDT DLILEVEHDC TTYGEEIKFG GGKTIRDGMS QTNSPSSYEL

        70         80         90        100        110        120
DLVLTNALIV DYTGIYKADI GIKDGKIAGI GKAGNKDMQD GVDNNLCVGP ATEALAAEGL

       130        140        150        160        170        180
IVTAGGIDTH IHFISPQQIP TAFASGVTTM IGGGTGPADG TNATTITPGR ANLKSMLRAA

       190        200        210        220        230        240
EEYAMNLGFL AKGNVSYEPS LRDQIEAGAI GFKIHEDWGS TPAAIHHCLN VADEYDVQVA

IHT
```

Q9RGP5 in FASTA format

View entry in original UniProtKB/TrEMBL format
View entry in raw text format (no links)
Request for annotation of this UniProtKB/TrEMBL entry

UniProtKB/TrEMBL entry Q9RGP5

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**Q9RGP5_HELFE**
Primary accession number	**Q9RGP5**
Secondary accession numbers	None
Entered in TrEMBL in	Release 13, May 2000
Sequence was last modified in	Release 13, May 2000
Annotations were last modified in	Release 26, March 2004

Name and origin of the protein

Protein name	**Urease [Fragment]**
Synonyms	None
Gene name	**Name: ureB**
From	Helicobacter felis [TaxID: 214]
Taxonomy	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.

References

[1] NUCLEOTIDE SEQUENCE.
STRAIN=ATCC51211;
Weir S.C., Stock F., Gill V.J., Fischer S.H.;
Submitted (DEC-1998) to the EMBL/GenBank/DDBJ databases.

Comments

None

Cross-references

EMBL	AF116580; AAF21996.1; -; Genomic_DNA.	[EMBL / GenBank / DDBJ] [CoDingSequence]
HSSP	P14917; 1E9Y. [HSSP ENTRY / PDB]	
SMR	Q9RGP5; 1-243.	
GO	GO:0016787; Molecular function: hydrolase activity *(inferred from electronic annotation).*	
	GO:0016151; Molecular function: nickel ion binding *(inferred from electronic annotation).*	
	GO:0009039; Molecular function: urease activity *(inferred from electronic annotation).*	
	GO:0006807; Biological process: nitrogen compound metabolism *(inferred from electronic annotation).*	
	QuickGo	

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Thursday, June 09, 2005

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND		
☐	L1	felis and urease	137
☐	L2	(felis and urease).ti,ab,clm.	29
	DB=USPT; PLUR=YES; OP=AND		
☐	L3	US-6248330-B1.did.	1

END OF SEARCH HISTORY

Generate Collection Print

Search Results - Record(s) 1 through 29 of 29 returned.

1. 20040138415. 03 Mar 04. 15 Jul 04. Helicobacter proteins, nucleic acids and uses thereof. Tian, Jing-Hui, et al. 530/350; C07K001/00 C07K014/00 C07K017/00.

2. 20040115772. 25 Nov 03. 17 Jun 04. Methods of inhibiting Helicobacter pylori. De Reuse, Hilde, et al. 435/69.1; C12Q001/68 C12P021/06.

3. 20040005325. 13 Jul 01. 08 Jan 04. Helicobacter felis vaccine. Kusters, Johannes Gerardus, et al. 424/184.1; A61K039/00 A61K039/38.

4. 20030007980. 18 Sep 01. 09 Jan 03. Urease-based vaccine and treatment for helicobacter infection. Michetti, Pierre, et al. 424/190.1; 424/234.1 A61K039/02.

5. 20020146423. 23 Dec 97. 10 Oct 02. PROTECTIVE HELICOBACTER ANTIGENS. DOIDGE, CHRISTOPHER VINCENT, et al. 424/184.1; A61K039/00 A61K039/38.

6. 20020107368. 06 Dec 00. 08 Aug 02. Helicobacter proteins, gene sequences and uses thereof. Tian, Jing-Hui, et al. 530/388.4; 424/190.1 530/350 536/23.7 A61K031/70 C07H021/04 C07K001/00 C07K014/00 C07K017/00 C07K016/00 C12P021/08.

7. 20020102269. 22 Dec 00. 01 Aug 02. Methods of inhibiting helicobacter pylori. De Reuse, Hilde, et al. 424/190.1; 435/32 435/7.32 A61K039/02 G01N033/554 G01N033/569 C12Q001/18.

8. 6468545. 20 Oct 99; 22 Oct 02. Treatment and prevention of helicobacter infection. Doidge; Christopher V., et al. 424/234.1; 435/252.1 435/34 435/69.1 536/23.4 536/23.5. A61K039/02.

9. 6416968. 23 Aug 00; 09 Jul 02. Methods of inhibiting Helicobacter pylori. De Reuse; Hilde, et al. 435/32; 424/141.1 424/150.1 424/184.1 424/234.1 424/236.1 424/94.1 435/106 435/12 435/18 435/252.1 435/29 435/4 435/6 435/69.1 514/230.5 514/44 530/300 530/350. C12Q001/18.

10. 6406703. 05 Jul 00; 18 Jun 02. Treatment of H. pylori associated gastroduodenal disease. Doidge; Christopher Vincent, et al. 424/234.1; 424/194.1 424/203.1 424/450 424/457 424/460 424/461 424/500 424/501 424/502 530/350. A61K039/02 A61K039/16 A61K009/64 A61K039/85 C07K001/00.

11. 6290962. 23 Feb 94; 18 Sep 01. Urease-based vaccine and treatment for helicobacter infection. Michetti; Pierre, et al. 424/185.1; 424/184.1 424/192.1 424/193.1 424/197.11 424/203.1 424/234.1 424/261.1 424/278.1 424/280.1 424/282.1 424/94.6 514/234.5 514/41. A61K039/00 A61K038/46 A61K031/70 A61K039/385.

12. 6258359. 06 Jun 95; 10 Jul 01. Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides. Labigne; Agnes, et al. 424/141.1; 424/150.1 424/163.1 424/164.1 530/350 530/388.1 530/388.2 530/388.4. A61K039/395 A61K039/40 C07K001/00 C07K016/00.

13. 6248330. 02 May 95; 19 Jun 01. Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides. Labigne; Agnes, et al. 424/192.1; 424/184.1 424/234.1 435/6 435/69.1. A61K039/00.

☐ 14. 6190667. 30 Jun 98; 20 Feb 01. Methods of inhibiting Helicobacter pylori. De Reuse; Hilde, et al. 424/234.1; 424/780 435/32. A61K039/02.

☐ 15. 6005090. 15 Aug 96; 21 Dec 99. Treatment and prevention of helicobacter infection. Doidge; Christopher V., et al. 536/23.5; 435/7.32. A61K039/02.

☐ 16. 5871749. 18 Aug 95; 16 Feb 99. Therapeutic treatment of H. pylori associated gastroduodenal disease. Doidge; Christopher Vincent, et al. 424/234.1; 424/194.1 435/7.21. A61K039/02.

☐ 17. 5843460. 06 Jun 95; 01 Dec 98. Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides. Labigne; Agnes, et al. 424/234.1; 435/6 435/7.32 435/7.9 514/234.5 514/41. A61K039/02.

☐ 18. JP02004337170A. 02 Jun 04. 02 Dec 04. IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES. LABIGNE, AGNES, et al. C12N015/09; A61K038/00 A61K039/00 A61K039/39 A61K039/395 A61P001/04 A61P035/00 A61P037/02 C07K014/205 C07K016/12 C07K016/40 C12N001/15 C12N001/19 C12N001/21 C12N005/10 C12N009/80 C12Q001/68 C12P021/08.

☐ 19. JP02002355054A. 16 Jul 01. 10 Dec 02. HELICOBACTER FELIS VACCINE. KUSTERS, JOHANNES GERARDUS, et al. C12N015/09; A61K038/00 A61K039/106 A61K039/118 A61K039/12 A61K039/175 A61K039/23 A61K039/235 A61K039/39 A61K039/395 A61P001/04 A61P031/04 C12N001/15 C12N001/19 C12N001/21 C12N005/10 C12N009/80 C12Q001/68 G01N033/15 G01N033/50 G01N033/53 G01N033/566 G01N033/569.

☐ 20. EP001176192A2. 11 Jul 01. 30 Jan 02. Helicobacter felis vaccine. KUSTERS, JOHANNES GERARDUS, et al. C12N009/80; C07K014/205 C07K016/12 C12Q001/68 A61K039/106 A61K048/00 G01N033/53 G01N033/68.

☐ 21. WO009634624A1. 02 May 96. 07 Nov 96. IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES. LABIGNE, AGNES, et al. A61K039/106; C07K016/12 A61K039/40.

☐ 22. WO009514093A1. 19 Nov 93. 26 May 95. IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES. LABIGNE, AGNES, et al. C12N015/31; C12N009/80 C12Q001/68 C12P021/08 A61K039/106.

☐ 23. WO009426901A1. 19 May 94. 24 Nov 94. IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES. LABIGNE, AGNES, et al. C12N015/31; C12N009/80 C12Q001/68 C12P021/08 A61K039/106 G01N033/577.

☐ 24. EP 1176192A. Novel Helicobacter felis urease X and Y subunit polypeptides, useful in the diagnosis of Helicobacter felis infections and in the preparation of vaccines. CATTOLI, G, et al. A61K038/00 A61K039/00 A61K039/106 A61K039/118 A61K039/12 A61K039/175 A61K039/23 A61K039/235 A61K039/38 A61K039/39 A61K039/395 A61K048/00 A61P001/04 A61P031/04 C07K014/195 C07K014/205 C07K016/12 C12N001/15 C12N001/19.C12N001/21 C12N005/10

C12N009/80 C12N015/09 C12N015/52 C12N015/55 C12Q001/68 G01N033/15 G01N033/50 G01N033/53 G01N033/566 G01N033/569 G01N033/68 C12N009/80 C12Q001/68 C12R001:01 C12R001:01.

☐ 25. WO 200152667A. Use of lactic acid bacteria strain(s), metabolites or fermented medium, having anti-Helicobacter activity in vitro, in preparation of composition for prophylaxis/treatment of gastric disorders in pets. BALLEVRE, O, et al. A23K000/00 A23K001/00 A23K001/16 A23K001/18 A61K035/74 A61K045/00 A61P001/04 A61P031/04.

☐ 26. WO 200003730A. Use of Lactobacillus-derived urease, as a vaccine for preventing or treating Helicobacter infection and conditions including gastric ulcer, gastritis and gastric cancer caused by the infection. PARK, J B. A61K039/07 C07K014/335.

☐ 27. WO 9911284A. Vaccine containing lactobacilli expressing urease peptide - for treating gastrointestinal disorders. TABAQCHALI, S, et al. A61K038/43 A61K039/02 C12N015/74.

☐ 28. WO 9634624A. New immunogenic compsn. contg. UreB and HspA antigens of Helicobacter - for treatment and prevention of esp. H pylori infection, also new antibodies specific for these antigens.. FERRERO, R L, et al. A61K039/106 A61K039/40 C07K016/12.

☐ 29. US 5843460A. DNA from Helicobacter pylori and Helicobacter felis - used to develop prods. for detection, treatment and prevention of Helicobacter infection. FERRERO, R, et al. A61K038/00 A61K039/00 A61K039/02 A61K039/106 A61K039/39 A61K039/395 A61K039/40 A61P001/04 A61P035/00 A61P037/02 C07H021/04 C07K001/00 C07K014/195 C07K014/205 C07K016/00 C07K016/12 C07K016/40 C12N001/15 C12N001/19 C12N001/21 C12N005/10 C12N009/80 C12N015/09 C12N015/31 C12P021/02 C12P021/08 C12Q001/68 G01N033/569 G01N033/574 G01N033/577.

Generate Collection Print

Terms	Documents
(felis and urease).ti,ab,clm.	29

Prev Page Next Page Go to Doc#

US006406703B1

(12) **United States Patent**
Doidge et al.

(10) **Patent No.: US 6,406,703 B1**
(45) **Date of Patent: *Jun. 18, 2002**

(54) **TREATMENT OF *H. PYLORI* ASSOCIATED GASTRODUODENAL DISEASE**

(75) Inventors: **Christopher Vincent Doidge**, Box Hill; **Adrian Lee**, Lane Cove, both of (AU)

(73) Assignees: **CSL Limited**, Victoria (AU); **The University of New South Wales**, New South Wales (AU)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/610,937**

(22) Filed: **Jul. 5, 2000**

Related U.S. Application Data

(63) Continuation of application No. 09/182,062, filed on Oct. 29, 1998, now Pat. No. 6,129,923, which is a continuation of application No. 08/464,854, filed as application No. PCT/AU94/00416 on Jul. 25, 1994, now Pat. No. 5,871,749.

(30) **Foreign Application Priority Data**

Jul. 27, 1993 (AU) 0157/93
Feb. 14, 1994 (AU) 3828/94

(51) **Int. Cl.**[7] **A61K 39/02**; A61K 39/16; A61K 9/64; A61K 39/85; C07K 1/00

(52) **U.S. Cl.** **424/234.1**; 424/203.1; 424/460; 424/461; 424/450; 424/457; 424/500; 424/194.1; 424/501; 424/502; 530/350

(58) **Field of Search** 530/350; 424/234.1, 424/194.1, 500, 501, 502, 460, 461, 450, 457, 203.1; 435/7.21

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,370,872	A	12/1994	Cryz et al.	424/194.1
5,403,924	A	4/1995	Cover et al.	536/23.1
5,459,041	A	10/1995	Blaser et al.	435/7.21
5,538,729	A	7/1996	Czinn et al.	424/234.1
5,871,749	A *	2/1999	Doidge et al.	434/234.1
6,129,923	A *	10/2000	Doidge et al.	424/234.1
6,290,991	B1 *	9/2001	Roser et al.	424/502

FOREIGN PATENT DOCUMENTS

DE	4139840	6/1992
WO	90/04030	4/1990
WO	92/19970	11/1992
WO	93/16723	9/1993
WO	93/18150	9/1993
WO	93/20843	10/1993
WO	94/09823	5/1994
WO	95/22987	8/1995
WO	96/38475	12/1996
WO	98/18323	5/1998

OTHER PUBLICATIONS

Monath, TP etal, American Journal of Gastroenterology, vol. 89(8), p. 1383, 1994.*
McNulty, CA et al, European Journal of Clinical Microbiology and Infectious Diseases, Aug. 1988, vol. 7(4), pp. 566–569, (abstract only).*
Unge, P et al, Scandinavian Journal of gastroenterology, vol. 157, supplement, pp. 12–15, discussion 21–2, (abstract only).*
Guy, B et al, Vaccine, May 1988, vol. 16(8), pp. 850–856.*
Freland, C et al, Patholgie–biologie, Jun. 1987, vol. 35 (5 part 2), pp. 809–812,(abstract only).*
Freland, C et al, Pathologie–biologie, Sep. 1987, vol. 35 (7), pp. 1037–1042, (abstract only).*
McNulty, CA etal, Journal of antimicrobial chemotherapy, Nov. 1988, vol. 22(5), pp. 729–738, (abstract only).*
McNulty, CA et al, Antimicrobial agents and chemotherapy, Dec. 1985, vol. 28(6), pp. 837–838, (abstract only).*
Megraud, F et al, Antimicrobial agents and chemotherapy, May 1991, vol. 35(5), p. 869–872 (abstract only).*
Graham, DY et al, American Journal of Gastroenterology, Mar. 1989, vol. 84(3), apges 233–238 (abstract only).*
Lambert, T et al, Antimicrobial agents and chemotherapy, vol. 30(3), p. 510–511, (abstract only).*
Andreasen, JJ et al, ACTA pathologica, microbiologica et immunologica Scandinavica, Section B, Microbiology, Apr. 1987, vol. 95(2), pp. 147–149 (abstract only).*
Glupczynski, Y et al, American Journal of Gastroenterolgoy, Dec. 1990, vol. 85(12), pp. 1545–1551 (abstract only).*
Grayson, ML et al, European Journal of Clinical Microbiology and Infectious Diseases, vol. 8(10), apges 888–889, Oct. 1989(abstract only).*
Mertens, JC et al, Antimicrobial agents and chemotherapy, Feb. 1989, vol. 33(2), pp. 256–257, (abstract only).*
Alarcon, T et al, International Journal of antimicrobial agents, Jun. 1999, vol. 12(1), apges 19–26, (abstract only).*
Czinn et al., "Infection and Immunity"; 59(7), pp. 2359–2363, (1991).
Czinn et al., "Vaccine"; 11(6), pp. 637–642, (1993).
E. Dick–Hegedus et al., "Scand J Gastroenterol"; 26, pp. 909–915, (1991).
Goodwin et al., "*Helicobacter Pylori* Biology and Clinical Practice", pp. 432–444 (1993).
Lee et al., "Gastroenterology"; 99, pp. 1315–1323, (1990).
O'Connor, "Postgrad Med J."; 68, pp. 549–557, (1992).
Parsonnet et al., "The New England Journal of Medicine"; 325(16), pp. 1127–1131, (1991).

(List continued on next page.)

Primary Examiner—Lynette R. F. Smith
Assistant Examiner—Ginny Allen Portner
(74) *Attorney, Agent, or Firm*—Foley & Lardner

(57) **ABSTRACT**

The application discloses a method for the treatment of Heliobacter infection in a mammalian host, which comprises administration to said infected host of an immunologically effective amount of one or more Heliobacter antigen(s), optionally in association with a mucosal adjuvant.

41 Claims, No Drawings

OTHER PUBLICATIONS

Laszlo et al., "Orvosi Hetilap"; 133, *Evfolyam*; 6, Szam, pp. 359–361.

Burke, "Vaccine"; 11(8), pp. 795–804, (1993).

Cox et al., "Animal Parasite Control Utilizing Biotechnology", pp. 49–112, (1992).

Blaser, "Clinical Infectious Diseases"; 15, pp. 386–393, (1992).

McGhee et al., "Infectious Agents & Disease 2", *New Prospectives in Vaccine Development:Mucosal*, Immunity to Infections, pp. 55–73 (1993).

Walker, "Vaccine", New Strategies for Using Mucosal Vaccination to Achieve More Effective, *Immunization*, 12(5), pp. 387–400, (1994).

Ghiara et al. "Infaction & Immunity", Therapeutic Intragastric Vaccination Against *Helicobacter Pylori* in *Mice Eradicates an Otherwise*. . . ; 65(12), pp. 4996–5002, (1997).

Michetti et al. "Gatroenterology", Oral Immunization of *H. pylori* Infected Adults with Recombinant Urease and LT Adjuvant 112(4), (1997) Abstract.

Corthesy–Theulaz, "Gastroenterology", Oral Umminization with *H. Pylori* Urease B Subunitas Treatment Against Heliobacter Infection in Mice, 109, pp. 115–121, (1995).

K. Heap., et al., "Microbiol. Ecol. Haelth Dis.", vol. 4, p.S148, (10/7–10/1991).

M.L. Dunkley et al., "Microbiol. Ecol. Health Dis.", vol. 4 (Spec. issue), p. S148, (1991).

C. Davin et al., "Gastroenterology", vol. 104(4), p. A1668, (Apr. 1993).

V. Laszlo et al., "Orvosi Hetilap", vol. 133(6), pp. 359–361, (1992).

M. Chen et al., "Gastroenterology", vol. 104(4), p. A681, (Apr. 1993).

"Genesis Report—DX", ISSN, pp. 1061–2289, (Jan. 1993).

M. Chen et al., "FEMS Microbiolog. Letters", vol. 116, pp. 245–250.

"HP Worldwide Quarterly", pp. 1–7, (1991–1992).

R. Rappuoli et al., "Eur. J. Gastroenterol. & Hepatol.", vol. 5 (Suppl. 2), pp. S76–S78, (1993).

Czinn et al., "Gastroenterology", vol. 100 (5 pt. 2), p. A571, (May 1991).

J.R. McGhee et al., "Vaccine ", vol. 10(2), p. 75–88,(1992).

J.D. Clements et al., "Vaccine", vol. 6(3), pp. 269–277, (Jun. 1988).

O. R. Pavlovskis et al., "American Soc. Microbiol.", #E11, p. 119 (5–9, 1991).

C. K. Lee et al., "J of Infect. Disease", vol. 172, pp. 161–172, (1995).

Kaplan, L.; South African Medical Journal, vol. 83(12), pp. 922–923, Dec.

* cited by examiner

TREATMENT OF *H. PYLORI* ASSOCIATED GASTRODUODENAL DISEASE

This application is a continuation of application Ser. No. 09/182,062, filed Oct. 29, 1998, now U.S. Pat. No. 6,129,923 which is a continuation of application Ser. No. 08/464,854 filed Aug. 18, 1995, now U.S. Pat. No. 5,871,749, which is a national stage of PCT/AU94/00416 FILED Jul. 25, 1994.

FIELD OF THE INVENTION

This invention relates to the treatment of gastroduodenal disease associated with *Helicobacter pylori* infection and in particular it relates to the use of active immunisation as a treatment for *H. pylori*—associated gastroduodenal disease.

BACKGROUND OF THE INVENTION

The bacterium, *Helicobacter pylori,* is now well established as a major gastroduodenal pathogen, and more than 50% of the world population is infected with this organism which causes gastritis of varying severity. While no symptoms are apparent in a great proportion of infected persons, in a significant number of *H. pylori* infected persons overt disease may result. The majority (95%) of duodenal ulcers are associated with *H. pylori* infection; a causal role is shown by treatment studies which indicate that if the organisms can be eradicated at the time of ulcer healing then the ulcers do not recur—in contrast to 80% recurrence rate at one year in those who remain infected with the organisms. Furthermore, up to 80% of gastric ulcers are thought to be *H. pylori* associated (Blaser, 1992).

There is now increasing evidence of the harmful consequence of long term *H. pylori* infection. In countries such as China, Colombia and Japan the bacterium is picked up very early in life, and in these persons the gastritis slowly progresses until after 30–40 years of continual infection, severe gastric atrophy appears. Gastric atrophy is well documented as being the precursor lesion for gastric cancer, although the actual cancer that develops in an atrophied stomach is dependent on a myriad of other factors including diet. However, all the evidence to date would suggest that the cancer would not develop if it was possible to remove the *H. pylori* infection at an early age before the atrophy had developed (Parsonnet et al., 1991).

There is no laboratory animal model of *H. pylori* infection that can be used for large scale assessment of new anti-*H. pylori* therapies. However, a *Helicobacter felis* mouse model of gastric Helicobacter infection has been developed that has proved extremely useful in the screening of the potential of new antimicrobial therapeutic regimens. *H. felis* is a spiral shaped bacterium that is very closely related to *H. pylori.* This bacterium colonises the stomach of mice in a very similar way to *H. pylori* in the human, i.e. the main ecological niche is gastric mucus and the localisation of colonisation is antral dominant. In germfree mice, *H. felis* infection induces a gastritis that is very similar to the human *H. pylori* infection with a chronic inflammation accompanied by polymorphonuclear leucocyte infiltration. Infection with each organism results in the induction of a similar raised immune response against *H. pylori* and *H. felis* respectively (Lee et al., 1990).

The *H. felis* mouse model has proved to be very predictive of the efficacy of anti-*H. pylori* agents in humans. Thus, monotherapy with agents with high in vitro activity such as erythromycin show no significant in vivo effect against *H. felis* in mice, just as erythromycin has no anti-*H. pylori* effect in humans despite high antimicrobial effects in vitro. In contrast, the triple therapy regimens of a bismuth compound, metronidazole, and tetracycline or amoxycillin lead to a very high eradication rate in *H. felis* infected mice (Dick-Hegedus and Lee, 1991). Such triple therapies are the most successful human anti-*H. pylori* regimens, and at the present time are recommended as the first choice for anti-*H. pylori* therapy. However, established Helicobacter infections are difficult to treat, and current chemotherapeutic regimens remain suboptimal due to problems with efficacy, toxicity, drug resistance and reinfection (O'Connor, 1992).

Active immunisation of already infected patients has not been proven efficacious for any clinically manifest human infectious disease (Burke, 1992). Given that *H. pylori* infections persist for long periods, if not the life of the infected individual, despite the presence of a vigorous immune response that includes a high level of circulating IgG antibody in the serum and the demonstration of local specific IgA antibody in the gastric mucosa, it has been considered that active immunisation was unlikely to be effective in therapy (Goodwin, 1993). Indeed, Czinn et al. (1993) in proposing that oral vaccination may be a feasible approach for the prevention of *H. pylori* infection in humans (based on an evaluation of an oral immunisation protocol in the *H. felis* mouse model), suggested that once infection is established neither antibody nor antibiotics are very effective at eradication.

Varga et al. (1992) have reported that a *H. pylori* vaccine prepared from organisms derived from a patient, and injected parenterally into that patient, resulted in an allergic reaction and failure to eradicate the organism.

Surprisingly, it has now been discovered for the first time that there is indeed a therapeutic potential for active immunisation against gastric Helicobacter infection. Furthermore, it has been discovered that oral administration of *H. pylori* antigen, with a suitable mucosal adjuvant, does not result in allergic or hypersensitivity symptoms, but results in suppression or eradication of the infecting organisms from the gastric mucosa.

SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided a method for the treatment of Helicobacter infection in a mammalian host, which comprises the oral administration to said infected host of an immunologically effective amount of one or more Helicobacter antigen(s), optionally in association with a mucosal adjuvant.

In another aspect, there is provided a vaccine composition for the treatment of Helicobacter infection in a mammalian host, which comprises an immunologically effective amount of one or more Helicobacter antigen(s), optionally in association with a mucosal adjuvant.

In yet another aspect, the present invention provides the use of a vaccine composition comprising an immunologically effective amount of one or more Helicobacter antigen (s), optionally in association with a mucosal adjuvant. in the treatment of Helicobacter infection in a mammalian host.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

By use of the term "immunologically effective amount" herein, it is meant that the administration of that amount to an individual infected host, either in a single dose or as part

of a series, is effective for treatment of Helicobacter infection. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the assessment of the medical situation, and other relevant factors. It is expected that the amount will all in a relatively broad range that can be determined through routine trials.

DETAILED DESCRIPTION OF THE INVENTION

The Helicobacter antigen(s) used in accordance with the present invention may be *H. felis* antigen(s), or more preferably *H. pylori* antigen(s). In a particularly preferred aspect of the present invention, a vaccine composition comprising *H. pylori* antigen(s) in association with a mucosal adjuvant is used the treatment of *H. pylori* infection in a human patient.

Preferably, the Helicobacter antigen(s) comprise a bacterial sonicate, and in particular a *H. pylori* sonicate. More preferably, the Helicobacter antigen(s) used in accordance with the present invention comprise inactivated whole bacterial cells of *H. pylori*.

Alternatively, the Helicobacter antigen(s) used in accordance with the present invention may comprise one or more individual antigens, particularly one or more *H. pylori* antigens such as *H. pylori* urease, or *H. pylori* cytotoxin (CT), Cytotoxin Associated Immunodominant (CAI) antigen or heat shock protein (hsp) as disclosed by way of example in International Patent Publication No. WO 93/18150.

One mucosal adjuvant which is optionally, and preferably, administered with the Helicobacter antigen(s) to the infected host is cholera toxin. Another preferred mucosal adjuvant which may be administered with the Helicobacter antigen(s) is *E. coli* heat labile toxin (*E. coli* HLT). Mucosal adjuvants other than cholera toxin and *E. coli* HLT which may be used in accordance with the present invention include non-toxic derivatives of cholera toxin, such as the B sub-unit (CTB), chemically modified cholera toxin, or related proteins produced by modification of the cholera toxin amino acid sequence. Each of these molecules with mucosal adjuvant or delivery properties may be added to, or conjugated with, the Helicobacter antigen(s). Other compounds with mucosal adjuvant or delivery activity may be used, such as: bile; polycations such as DEAE-dextran and polyornithine; detergents such as sodium dodecyl benzene sulphate; lipid-conjugated materials; antibiotics such as streptomycin; vitamin A; and other compounds that alter the structural or functional integrity of mucosal surfaces. Other mucosally active compounds include derivatives of microbial structures such as MDP; acridine and cimetidine.

Helicobacter antigen(s) may be delivered in accordance with this invention in ISCOMS (immune stimulating complexes), ISCOMS containing CTB, liposomes or encapsulated in compounds such as acrylates or poly(DL-lactide-co-glycoside) to form microspheres of a size suited to adsorption by M cells. Alternatively, micro or nanoparticles may be covalently attached to molecules such as vitamin B12 which have specific gut receptors. Antigen(s) may also be incorporated into oily emulsions and delivered orally. Ail extensive though not exhaustive list of adjuvants can be found in Cox and Coulter, 1992.

Other adjuvants, as well as conventional pharmaceutically acceptable carriers, excipients, buffers or diluents, may also be included in the therapeutic vaccine composition of this invention. The vaccine composition may, for example, be formulated in enteric coated gelatine capsules including sodium bicarbonate buffers together with the Helicobacter antigen(s) and mucosal adjuvant.

Generally, a vaccine composition in accordance with the present invention will comprise an immunologically effective amount of Helicobacter antigen(s), and optionally a mucosal adjuvant, in conjunction with one or more conventional pharmaceutically acceptable carriers and/or diluents. As used herein "pharmaceutically acceptable carriers and/or diluents" include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art and is described by way of example in *Remington's Pharmaceutical Sciences,* 18th Edition, 1990, Mack Publishing Company, Pennsylvania, U.S.A..

The pharmaceutical composition of this invention may be orally administered directly to the mammalian host, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatine capsule, or it may be compressed into tablets, or it may be incorporated directly with the solid or liquid food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of active component in the compositions and preparations may of course be varied and is such that a suitable dosage will be obtained to be immunologically effective.

Solid oral dosage units such as tablets. troches, pills, capsules and the like may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active component, sucrose as a sweetening agent, methyl and prophylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed.

The vaccine composition of the invention is administered orally in amounts readily determined by persons of ordinary skill in this art. Thus, for adults a suitable dosage would be in the range of 10 μg to 10 g, for example 50 μg to 3 g. Similar dosage ranges would be applicable for children.

As noted above, a suitable mucosal adjuvant is cholera toxin. The amount of mucosal adjuvant employed depends on the type of mucosal adjuvant used. For example, when the mucosal adjuvant is cholera toxin, it is suitably used in an amount of 10 nanogram to 50 μg, for example 01 μg to 10 μg. When the mucosal adjuvant is *E. coli* heat labile toxin, suitable amounts are 1 μg to 1 mg, for example 5 μg to 50 μg.

In work leading to the present invention, active immunisation of mice previously infected with *H. felis* , with oral

doses of cholera toxin or *E. coli* HLT adjuvant and a whole cell *H. felis* or *H. pylori* sonicate, result in the clearance of *H. felis* from the gastric mucosa. It is therefore anticipated that active immunisation of infected humans with oral doses of a mucosal adjuvant with *H. pylori* antigen(s) will result in the clearance of *H. pylori* from the gastric mucosa. Based on previous studies with this model using anti-*H. pylori* agents, it is considered that this is the first evidence of the therapeutic potential of active immunisation with *H. pylori* vaccines, and indicates that a vaccine composition for the therapy of human *H. pylori*—associated gastroduodenal disease is a preparation of Helicobacter antigen(s), optionally and preferably combined with a mucosal adjuvant.

It will be apparent to persons skilled in the field that effective treatment of *Helicobacter pylori* infection in humans with an oral vaccine composition of Helicobacter antigen(s) which will eradicate or suppress the infection will provide a significant therapeutic benefit via the suppression or elimination of gastritis, prevention of peptic ulcer relapse and reduction in the harmful sequelae of *Helicobacter pylori* infection including peptic ulceration and gastric cancer.

The present invention is further illustrated in the following, non-limiting Examples.

EXAMPLE 1

One hundred and sixty female SPF mice from the Animal Breeding Unit of the University of New South Wales, Australia, were infected with four oral doses of 10^9–10^{10} living *Helicobacter felis* (ATCC culture 49179) given two days apart.

Bacteria were grown in plastic Petri dishes on Blood Agar Base No. 2, 3.8% w/v (Oxoid, Basingstoke, U.K.) with 7% v/v whole horse blood (Oxoid), containing amphotericin B (Fungizone, Squibb, Princeton, N.J., USA) 2.5 mg/l; trimethoprim (Sigma, St.Louis, Mo., USA), 10 mg/l. Plates were incubated in a microaerophilic humid atmosphere (Oxoid, BRE56) at 37° C. for 48 hours.

Sonicates were prepared by growth of the organisms, as described above, followed by harvesting of the organisms in 0.1 molar phosphate buffered saline (PBS). The cells were washed, collected by centrifugation, washed once in PBS, and resuspended in fresh PBS. The cells were then sonicated at the rate of one minute per ml of cell suspension (50% duty cycle) using a B-30 Branson Cell Disrupter. The sonicate was stored at −20° C.

On days 28, 42, 44 and 47 after administration of the last infecting dose of *H. felis* , 20 of the mice were given orally 0.2 ml of a suspension containing 10 μg of cholera toxin (Sigma C 3012) and a sonicate of *H. felis* containing 1 mg protein (BIO-RAD DC protein assay).

Samples of antral mucosa were tested for infection using a rapid microtitre urease test as described previously (Lee et al., 1990). This test has been validated as being highly predictive of *H. felis* gastric infection. Groups of 40 mice (20 vaccinates and 20 controls) were euthanased at intervals of 1 week, 1 month, 2 months and 3 months after the last dose of vaccine.

The results are shown in Table 1.

These results show that treatment of *H. felis* infected mice with an oral vaccine comprised of Helicobacter antigens and a mucosal adjuvant, results in cure of the infection in a significant proportion of mice. This effect is evident 1 week after cessation of therapy, and continues for at least 3 months, demonstrating that the mice have been cured of their infection.

TABLE 1

	Proportion of *H. felis* infected mice			
Immunisation	1 week	1 month	2 months	3 months
Nil	19/19	20/20	18/19	13/19
Sonicate plus CT	2/20	3/20	6/20	1/17
	P < 0.0001*	P < 0.0001	P < 0.05	P < 0.0001

*Fisher's exact test (two tailed).

EXAMPLE 2

One hundred female BALB/c mice from the Animal Breeding Unit of the University of New South Wales, Australia, were infected with 3 oral doses of 10^8 living *Helicobacter felis* (ATCC culture 49179) given 2 days apart, i.e. days 1, 3 and 5.

Bacteria were grown in plastic Petri dishes on Blood Agar Base No. 2, 3.8% w/v (Oxoid, Basingstoke, U.K.) with 7% v/v whole horse blood), (Oxoid), containing amphotericin B (Fungizone, Squibb, Princeton, N.J., USA) 2.5 mg/l; trimethoprim (Sigma, St.Louis, Mo., USA), 10 mg/l. Plates were incubated in a microaerophilic humid atmosphere (Oxoid, BR56) at 37° C. for 48 hours.

Sonicates were prepared by growth of the organisms, as described above, followed by harvesting of the organisms in 0.1 molar phosphate buffered saline (PBS). The cells were washed collected by centrifugation, washed once in PBS, and resuspended in fresh PBS. The cells were then sonicated at the rate of one per minute per ml of cell suspension (50% duty cycle) using a B-30 Branson Cell Disrupter. The sonicate was stored at −20° C.

On days 21, 35, 37, and 40 after administration of the last infecting dose of *H. felis,* 20 mice were each given orally 0.2 ml of a solution containing 10 ug of cholera toxin (Sigma C 3012), 20 mice were each given orally 0.2 ml of a suspension containing 10 ug of cholera toxin and a sonicate of *H. felis* containing 1 mg protein (BIO-RAD DC protein assay), 20 mice were each given orally 0.2 ml of a suspension containing a sonicate of *H. felis* containing 1 mg protein, 20 mice were each given orally 0.2 ml of a suspension containing 10 ug of cholera toxin and a sonicate of *H. pylori* (strain 921023) containing 1 mg protein, and 20 mice were not orally vaccinated.

One week after the final immunising dose all the mice were euthanased. Samples of antral mucosa were tested for infection using a rapid microtitre urease test as described previously (Lee et al., 1990). This test has been validated as being highly predictive of *H. felis* gastric infection.

The results are shown in Table 2.

These results show that oral administration of Helicobacter antigens derived from either *H. felis* , or *H. pylori* along with a mucosal adjuvant, will cure a significant portion of *H. felis* infected mice.

TABLE 2

Vaccine	Number of animals infected	Significance
Nil	16/20	
CT alone	15/20	N.S.
H. felis sonicate alone	12/20	N.S.
H. felis sonicate plus CT	8/19	P < 0.05*
H. pylori sonicate plus CT	4/20	P < 0.001

*Fisher's exact test (two tailed)

EXAMPLE 3

One hundred female SPF mice from the Animal Breeding Unit of the University of New South Wales, Australia, were

infected with 4 oral doses of 10^9–10^{10} living Helicobacter felis (ATCC culture 49179) given 2 days apart. 20 female SPF mice were left uninfected. as negative controls.

Bacteria were grown in plastic Petri dishes on Blood Agar Base No. 2, 3.8% w/v (Oxoid, Basingstoke, UK) with 7% v/v whole horse blood (Oxoid), containing amphotericin B (Fungizone, Squibb, Princeton, N.J., USA) 2.5 mg/l; trimethoprim (Sigma, St.Louis, Mo., USA), 10 mg/l. Plates were incubated in a microaerophilic humid atmosphere (Oxoid, BR56) at 37° C. for 48 hours.

Sonicates were prepared by growth of the organisms, as described above, followed by harvesting of the organisms in 0.1 molar phosphate buffered saline (PBS). The cells were washed, collected by centrifugation, washed once in PBS, and resuspended in fresh PBS. The cells were then sonicated at the rate of one per minute per ml of cell suspension (50% duty cycle) using a B-30 Branson Cell Disrupter. The sonicate was stored at −20° C.

Starting between 6 weeks and 9 weeks after their last infecting dose of *H. felis*, 20 mice were each given orally 0.2 ml of a solution containing 25 μg of *E. coli* heat labile toxin (HLT) (Sigma E 8015), 20 mice were each given orally 0.2 ml of a suspension containing 25 μg of HLT and a sonicate of *H. pylori* containing 1 mg protein (BIO-RAD DC protein assay), 20 mice were each given orally 0.2 ml of a suspension containing a sonicate of *H. pylori* containing 1 mg protein, and 40 mice were not orally vaccinated.

Each group received three further doses 15, 17 and 20 days after their initial dose.

Four weeks after the final immunising dose all the mice were euthanased. Samples of antral mucosa were tested for infection using a rapid microtitre urease test as described previously (Lee et al., 1990). This test has been validated as being highly predictive of *H. felis* gastric infection.

The results are shown in Table 3.

They show that oral administration of Helicobacter antigens derived from *H. pylori* along with a mucosal adjuvant *E. coli* heat labile toxin, will cure a significant portion of *H. felis* infected mice.

TABLE 3

Treatment Group	Proportion of *H. felis* infected mice
Uninfected, unvaccinated	0/20
Infected, unvaccinated	40/40
Infected, Hp antigen alone	20/20
Infected, *E. coli* HLT alone	20/20
Infected, Hp antigen & HLT	6/19*

*P < 0.0001 (Fisher's exact test, two tailed).

REFERENCES

Blaser, M. J. (1992). *Helicobacterpylori:* Its role in disease. *Clin. Infect. Dis.* 15, 386–393.

Burke, D. S. (1993). Of postulates and peccadilloes: Robert Koch and vaccine (tuberculin) therapy for tuberculosis. *Vaccine,* 11, 795–804.

Cox, J. and Coulter, A. (1992). Advances in Adjuvant Technology and Application. In Animal Parasite Control Utilising Biotechnology. Edited W. K. Yong, CRC Press.

Czinn, S. J., Cai, A. and Nedrud, J. G. (1993). Protection of germ-free mice from infection by *Helicobacter felis* after active oral or passive IgA immunization. *Vaccine,* 11, 637–642.

Dick-Hegedus, E. and Lee, A. (1991). Use of a mouse model to examine anti-*Helicobacter pylori* agents. *Scand. J. Gastrolenterol.* 26, 909–915.

Goodwin, C. S. (1993). Overview of *Helicobacter pylori* gastritis, peptic ulcer, and gastric cancer and the possible development of an *H. pylori* vaccine. *In Helicobactor pylori* Biology and Clinical Practice. Edited by Goodwin and Worsley. CRC Press.

Lee, A., Fox, J. G., Otto, G. and Murphy, J. (1990). A small animal model of human *Helicobacter pylodi* active chronic gastritis. *Gastroenterology,* 99, 1316–1323.

O'Connor, H. J. 91992). Eradication of *Helicobacter pylori:* Therapies and clinical implications. *Postgrad. Med. J.* 68, 549–557.

Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelman, H. J., Orentreich, N. and Sibley, R. K. (1991). *Helicobacter pylori* infection and the risk of gastric carcinoma. *N. Engl. J. Med.* 325, 1127–1131.

Varga, L., Löcsei, Z., Döbrönte, Z., Lakatos, F., Brözik, M. and Meretey, K. (1992). *Helicobacter pylori* allergy. *Orv. Hetil.* 133, 359–361.

What is claimed is:

1. A method of producing a composition, the method comprising combining:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) a mucosal adjuvant;

(c) an antibiotic; and

(d) a pharmaceutically acceptable carrier or diluent;

thereby producing a composition that is effective for eradicating or suppressing a pre-existing Helicobacter infection in a mammalian host when administered to the host.

2. The method of claim 1, wherein said one or more Helicobacter antigens comprise one or more *H. pylori* antigens.

3. The method of claim 1, wherein said one or more Helicobacter antigens comprise one or more *H. felis* antigens.

4. The method of claim 1, wherein said one or more Helicobacter antigens are provided in a sonicate of Helicobacter cells.

5. The method of claim 1, wherein said adjuvant is cholera toxin, a non-toxic derivative of cholera toxin, or *E. coli* heat labile toxin.

6. The method of claim 1, wherein the mucosal adjuvant has mucosal delivery activity.

7. The method of claim 1, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

8. A composition comprising:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) an antibiotic;

(c) a mucosal adjuvant; and

(d) an immune stimulating complex (ISCOM);

wherein the composition is effective for eradicating or suppressing a pre-existing Helicobacter infection in a mammalian host when administered to the host.

9. A composition comprising:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) an antibiotic;

(c) a mucosal adjuvant; and

(d) a liposome;

wherein the composition is effective for eradicating or suppressing a pre-existing Helicobacter infection in a mammalian host when administered to the host.

10. A composition comprising:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) an antibiotic;

(c) a mucosal adjuvant; and

(d) a microsphere;

wherein the composition is effective for eradicating or suppressing a pre-existing Helicobacter infection in a mammalian host when administered to the host.

11. A composition comprising:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) an antibiotic;

(c) a mucosal adjuvant; and

(d) an oily emulsion;

wherein the composition is effective for eradicating or suppressing a pre-existing Helicobacter infection in a mammalian host when administered to the host.

12. A composition comprising:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) an antibiotic;

(c) a mucosal adjuvant; and

(d) an enteric coated gelatin capsule;

wherein the composition is effective for eradicating or suppressing a pre-existing Helicobacter infection in a mammalian host when administered to the host.

13. A composition comprising:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) an antibiotic; and

(c) a pharmaceutically acceptable carrier or diluent,

wherein the composition is effective for eradicating or suppressing a pre-existing Helicobacter infection in a mammalian host when administered to the host.

14. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising administration of the composition of claim 8 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

15. The method of claim 14, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

16. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration of the composition of claim 8 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

17. The method of claim 16, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

18. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising administration of the composition of claim 9 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

19. The method or claim herein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

20. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration of the composition of claim 9 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

21. The method of claim 20, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

22. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising administration of the composition of claim 10 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

23. The method of claim 22, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

24. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration of the composition of claim 10 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

25. The method of claim 24, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

26. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising administration of the composition of claim 11 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

27. The method of claim 26, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

28. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration of the composition of claim 11 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

29. The method of claim 28, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

30. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising administration of the composition of claim 12 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

31. The method of claim 30, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* i cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

32. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration of the composition of claim 12 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

33. The method of claim 28, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

34. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising administration of the composition of claim 13 to a mucosal surface of the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

35. The method of claim 32, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

36. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration of the composition of claim 13 to the infected host, wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

37. The method of claim 36, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

38. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising administration to a mucosal surface of the infected host of a composition comprising:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) an antibiotic;

(c) a mucosal adjuvant and

(d) a pharmaceutically acceptable carrier or diluent;

wherein the administration of the composition eradicates or suppresses the pre-existing infection in the host.

39. The method of claim 38, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

40. A method for the treatment of a pre-existing Helicobacter infection in a mammalian host, the method comprising oral administration to the infected host of a composition comprising:

(a) an immunologically effective amount of one or more Helicobacter antigens;

(b) an antibiotic;

(c) a mucosal adjuvant and

(d) a pharmaceutically acceptable carrier or diluent;

wherein the administration of the composition eradicates or suppresses the preexisting infection in the host.

41. The method of claim 40, wherein said one or more Helicobacter antigens are selected from the group consisting of *H. pylori* urease, *H. pylori* cytotoxin, *H. pylori* cytotoxin associated immunodominant antigen, and *H. pylori* heat shock protein.

* * * * *

DOCUMENT-IDENTIFIER: US 6406703 B1
TITLE: Treatment of H. pylori associated gastroduodenal disease

CLAIMS:

3. The method of claim 1, wherein said one or more Helicobacter antigens comprise one or more H. felis antigens.

7. The method of claim 1, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

15. The method of claim 14, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

17. The method of claim 16, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

19. The method or claim herein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

21. The method of claim 20, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

23. The method of claim 22, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

25. The method of claim 24, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

27. The method of claim 26, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

29. The method of claim 28, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

31. The method of claim 30, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori i cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

33. The method of claim 28, wherein said one or more Helicobacter antigens are selected from the group

consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

35. The method of claim 32, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

37. The method of claim 36, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

39. The method of claim 38, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

41. The method of claim 40, wherein said one or more Helicobacter antigens are selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

DOCUMENT-IDENTIFIER: US 6290962 B1
** See image for Certificate of Correction **
TITLE: Urease-based vaccine and treatment for helicobacter infection

Abstract Text (1):
Method of eliciting in a mammalian host a protective immune response to Helicobacter infection and treatment of Helicobacter infection by administering to the host an immunogenically effective amount of a Helicobacter urease or urease subunits as antigen. Vaccine compositions are also provided.

CLAIMS:

1. A method of treating Helicobacter infection in a mammal, said method comprising administering to a mucosal surface of said mammal a therapeutically effective amount of a purified polypeptide comprising an A subunit of a naturally occurring Helicobacter urease.

3. The method of claim 1, wherein said Helicobacter urease is Helicobacter pylori urease.

21. A method of treating Helicobacter infection in a mammal, said method comprising administering to a mucosal surface of said mammal a therapeutically effective amount of a purified polypeptide comprising a B subunit of a naturally occurring Helicobacter urease.

23. The method of claim 21, wherein said Helicobacter urease is Helicobacter pylori urease.

41. A vaccine composition consisting essentially of a polypeptide comprising an A subunit of a naturally occurring Helicobacter urease, and a mucosal adjuvant.

42. The vaccine composition of claim 41, wherein said Helicobacter urease is Helicobacter pylori urease.

43. The vaccine composition of claim 41, wherein said Helicobacter urease is Helicobacter felis urease.

54. A vaccine composition comprising a purified polypeptide comprising an A subunit of a naturally occurring Helicobacter urease, and a polypeptide of the labile toxin of Escherichia coli.

56. A vaccine composition consisting essentially of a polypeptide comprising a B subunit of a naturally occurring Helicobacter urease, and a mucosal adjuvant.

57. The vaccine composition of claim 56, wherein said Helicobacter urease is Helicobacter pylori urease.

58. The vaccine composition of claim 56, wherein said Helicobacter urease is Helicobacter felis urease.

69. A vaccine composition comprising a purified polypeptide comprising a B subunit of a naturally occurring Helicobacter urease, and a polypeptide of the labile toxin of Escherichia coli.

US-PAT-NO: 6290962
DOCUMENT-IDENTIFIER: US 6290962 B1
**** See image for Certificate of Correction ****

TITLE: Urease-based vaccine and treatment for helicobacter infection

DATE-ISSUED: September 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Michetti; Pierre	Lausanne			CH
Corthesy-Theulaz; Irene	Lausanne			CH
Blum; Andre	Romammotier			CH
Davin; Catherine	Nyon			CH
Haas; Rainier	Tubingen			CH
Kraehenbuhl; Jean-Pierre	Rivat			CH
Saraga; Emilia	Lausanne			CH

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
OraVax, Inc.	Cambridge	MA			02

APPL-NO: 08/ 200346 [PALM]
DATE FILED: February 23, 1994

PARENT-CASE:
RELATED APPLICATIONS This application is a continuation-in-part of U.S. application Ser. No. 08/085,938, filed Jul. 6, 1993 now U.S. Pat. No. 5,972,336, which is a continuation-in-part application of U.S. application Ser. No. 07/970,996, filed Nov. 3, 1992, now abandoned the whole of which applications (including drawings) are hereby incorporated by reference.

INT-CL: [07] A61 K 39/00, A61 K 38/46, A61 K 31/70, A61 K 39/385

US-CL-ISSUED: 424/185.1; 424/234.1, 424/184.1, 424/192.1, 424/193.1, 424/197.1, 424/261.1, 424/280.1, 424/278.1, 424/94.6, 424/282.1, 424/203.1, 514/41, 514/234.5
US-CL-CURRENT: 424/185.1; 424/184.1, 424/192.1, 424/193.1, 424/197.11, 424/203.1, 424/234.1, 424/261.1, 424/278.1, 424/280.1, 424/282.1, 424/94.6, 514/234.5, 514/41

FIELD-OF-SEARCH: 424/234.1, 424/192.1, 424/193.1, 424/197.11, 424/261.1, 424/278.1, 424/280.1, 424/185.1, 424/184.1, 424/94.6, 424/282.1, 424/203.1, 514/41, 514/234.5

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4879213	November 1989	Fox et al.	
☐	5403924	April 1995	Cover et al.	536/23.1
☐	5538729	July 1996	Czinn et al.	
☐	5578302	November 1996	Brassart et al.	
☐	5733740	March 1998	Cover et al.	435/7.32
☐	5859219	January 1999	Cover et al.	536/22.1
☐	5871749	February 1999	Doidge et al.	424/234.1
☐	5972336	October 1999	Michetti et al.	
☐	5985631	November 1999	Soman et al.	
☐	6060241	May 2000	Corthesy-Theulaz	
☐	6096521	August 2000	Haas et al.	

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO A 91/16072	October 1991	WO	
WO 93/16723	February 1993	WO	
WO 94/09823	November 1994	WO	
WO 95/03824	February 1995	WO	

OTHER PUBLICATIONS

Solnick et al, Infection and Immunity 68/5: 2560-565, 2000.*
Corthesy-Theulaz et al, Gastroenterology 109: 115-121, 1995.*
Sutton et al, Vaccine 18: 2677-2685, 2000.*
Weltzin et al, Injection and Immunity 68/5: 2775-782 2000.*
Michetti et al, Gastroenterology 116: 804-812, 1999.*
"Vaccines" (Plotkin, S.A ed) see pp. 570, and 571, published by W.B. Saunders Company, 1988.*
Davin et al., "H. Pylor Urease Elicits Protection Against H. felisinfection in Mice, " Gastroenterology 104: A 1035 (1993).
Ferrero et al., "Recombinant Antigens Prepared from the Urease Subunits of Helicobacterspp: Evidence of Protection in a Mouse Model of Gastric Infection, " Infection and Immunity 62: 4981-4989 (1994).
Michetti et al., "Immunization of BALB/c Mice Against Helicobacter felisInfection With Helicobacter pyloriUrease, " Gastroenterolgy 107: 1002-1011 (1994).
Cornelissen et al., Anti-idiotypic Immunization Provides Protection Against Lethal Endotoxaemia in BALB/c Mice, Immunology 79:673-680, 1993.
Isaacson, Pathegenes is and Early Lesions in Extranodal Lymphoma, Toxicology Letters 67:237-247, 1993.
Kingsman and Kingsman, Polyvalent Recombinant Antigens: A New Vaccine Strategy, Vaccine 6:304-306, 1988.
Schmitt et al., Degradation and Release Properties of Pellets Fabricated from Three Commercial Poly(D, L-lactode-co-glycolide) Biodegradable Polymers, J. Pharmaceutical Sciences 82:326-329, 1993.

Sobala et al., Levels of Nitrite, Nitrute, N-nitroso Compound, Ascorbic Acid and total Bile Acids in Gastric Juice of Patients With and Without Precancerous Conditions of the Stomach, Carecinogenesis 12:193-198, 1991.

ART-UNIT: 165

PRIMARY-EXAMINER: Mannfield; Nita

ATTY-AGENT-FIRM: Clark & Elbing LLP

ABSTRACT:

Method of eliciting in a mammalian host a protective immune response to Helicobacter infection and treatment of Helicobacter infection by administering to the host an immunogenically effective amount of a Helicobacter urease or urease subunits as antigen. Vaccine compositions are also provided.

72 Claims, 10 Drawing figures

DOCUMENT-IDENTIFIER: US 6258359 B1
** See image for Certificate of Correction **
TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):
i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori (SEQ ID NO: 22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter felis urease (SEQ ID NO: 20-21), and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis (SEQ ID NO: 20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter pylori urease (SEQ ID NO: 22-26);

CLAIMS:

4. A composition comprising purified monoclonal antibodies directed against the following peptides:

a) at least one urease polypeptide of Helicobacter felis or Helicobacter pylori selected from the group consisting of UreA, UreB, UreE, UreF, UreG, UreH, UreI, and fragments thereof, wherein said fragment is also recognized by an antibody directed against the full length polypeptide corresponding to that fragment; and

b) at least one polypeptide of Helicobacter felis or Helicobacter pylori selected from the group consisting of HspA (SEQ ID NO: 29), HspB (SEQ ID NO: 30), and fragments thereof, wherein said fragment is also recognized by an antibody directed against the full length polypeptide corresponding to that fragment.

5. The composition of claim 4, wherein said urease polypeptide is UreA of Helicobacter pylori.

6. The composition of claim 4, wherein said urease polypeptide is UreB of Helicobacter pylori.

7. The composition of claim 4, wherein said urease polypeptides are UreA and UreB of Helicobacter pylori.

8. The composition of claim 4, wherein said urease polypeptide is UreA of Helicobacter felis.

9. The composition of claim 4, wherein said urease polypeptide is UreB of Helicobacter felis.

10. The composition of claim 4, wherein said urease polypeptides are UreA and UreB of Helicobacter felis.

US-PAT-NO: 6258359
DOCUMENT-IDENTIFIER: US 6258359 B1
** See image for Certificate of Correction **

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

DATE-ISSUED: July 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Labigne; Agnes	Bures sur Yvette			FR
Suerbaum; Sebastian	Veitshochheim			DE
Ferrero; Richard L.	Paris			FR
Thiberge; Jean-Michel	Plaisir			FR

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Institut Pasteur	Paris			FR	03

APPL-NO: 08/ 466248 [PALM]
DATE FILED: June 6, 1995

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a divisional application of application Ser. No. 08/447,177, filed May 19, 1995, now abandoned, which is a continuation-in-part of application Ser. No. 08/432,697, filed May 2, 1995, which is a continuation-in-part of International Application PCT/EP94/01625, filed May 19, 1994, which is based on International Application PCT/EP93/03259, filed Nov. 19, 1993, and European Application No. 93 401 309.5, filed May 19, 1993. Applicants claim the benefits of the International filing dates and priority of the European filing date. The entire disclosure of each of these applications is relied upon and incorporated by reference herein.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	93 401 309	May 19, 1993
WO	PCT/EP93/03259	November 19, 1993

INT-CL: [07] A61 K 39/395, A61 K 39/40, C07 K 1/00, C07 K 16/00

US-CL-ISSUED: 424/141.1; 424/150.1, 424/163.1, 424/164.1, 530/350, 530/388.1, 530/388.2, 530/388.4
US-CL-CURRENT: 424/141.1; 424/150.1, 424/163.1, 424/164.1, 530/350, 530/388.1, 530/388.2, 530/388.4

FIELD-OF-SEARCH: 424/234.1, 424/141.1, 424/150.1, 424/163.1, 424/164.1, 514/2, 530/350, 530/388.1, 530/388.2, 530/388.4

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5538729	July 1996	Czinn et al.	
☐	5858773	January 1999	Mazodier et al.	
☐	5972336	October 1999	Michetti et al.	

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 89/12455	December 1989	WO	
WOA9004030	April 1990	WO	
WOA9109049	June 1991	WO	
WOA9307273	April 1993	WO	
WOA9316723	September 1993	WO	
WOA9318150	September 1993	WO	
WOA9320843	October 1993	WO	
WOA9406474	March 1994	WO	
WOA9409823	May 1994	WO	
WOA9503824	February 1995	WO	
WO 9638475	December 1996	WO	
WO 9640893	December 1996	WO	

OTHER PUBLICATIONS

Roitt et al (Immunology, Grower Medical Publishing, London p. 601), 1985.*
Lazar et al. Molecular +Cellular Biology vol. 8 No. 3 pp 1247-1252, Mar. 1988.*
Burgess et al. J. of Cell Biology vol. 111 pp 2129-2138, Nov. 1990.*
Salgaller et al. Cancer Immunol. Immunother vol 39 pp 105-116, 1994.*
Smoot et al. Infection +Immunity vol. 58 No. 6 pp 1992-1994, Jun. 1990.*
Hu et al Infection +(Immunity vol. 60, No. 7, pp 2657-2666), Jul. 1992.*
Burns et al Journal of Biological Chemistry vol. 267 (36) pp 25632-25635, Dec. 25, 1992.*
Michetti et al (Gastroenterology vol. 107 pp 1002-1011), Oct. 1994.*
Hu et al (Infection+Immunity vol. 58 No 4. pp 992-998), Apr. 1990*
Sevier et al (Clinical Chemistry vol. 27 No. 11, pp 1797-1806, 1981).*
R.L. Ferrero et al., "Molecular Evidence Demonstrating Significant Homology Between the Urease Polypeptides of Helicobacter Felis and and Helicobacter Pylori," Gastroenterology , vol. 104, No. 4, Apr. 1993, Elsevier, New York, U.S.; P. A699.
E.G. Fox, et al. "Comparison of Two New Immunodiagnostic Assays for Helicobacter Pylori with Established Clinical and Histopathologic Findings", Gastroenterology, vol. 100, No. 5, Part 2, p. A66,

1991.
B.E. Dunn et al., "Identification and Purification of a cpn60 Heat Shock Protein Homolog from Helicobacter Pylori, " Infection and Immunity, vol. 60, No. 5, May 1992, Am. Soc. Microbiol., Baltimore, US; pp. 1946-1951.
D.J. Evans et al., "Urease-associated Heat Shock Protein of Helicobacter Pylori," Infection and Immunity, vol. 60, No. 5, May 1992, Am, Soc. Microbiol., Baltimore, US; pp. 2125-2127.
P.A. Foxall et al., "Use of Polymerase Chain Reaction-amplified Helicobacter Pylori Urease Structural Genes for Differentiation of Isolates" J. Clin. Microbiol., vol. 30, No. 3, Mar. 1992, AM. SOC. MICROBIOL, Washington, DC, US; pp. 739-741.
R.L. Ferrero and A. Labigne, "Cloning, Expression, and Sequencing of Helicobacter Felis Urease Genes, " Molec. Microbiol , vol. 9, No. 2, Jul. 14, 1993, Blackwell Sci. Pub., Oxford, UK; pp. 323-333.
S. Suerbaum and A. Labigne, "Cloning and Sequencing of the HspA and HspB heat shock protein encoding genes of Helicobacter pylori, " Abstr. Gen. Meet. Am. Soc. Microbiol., vol. 93, No. O, May 19, 1993, p. 127.
Suerbaum, Sebastian et al., "Helicobacter pylori hspA-hspB heat--shock gene cluster: nucleotide sequence, expression, putative function and immunogenicity" Molecular Microbiology (1994) 14(5), 959-974.
Richard L. Ferrero et al., "Recombinant Antigens Prepared from the Urease Subunits of Helicobacter spp. : Evidence of Protection in a Mouse Model of Gastric Infection," Infection and Immunity, Nov. 1994, p. 4981-4989.
J. Pappo et al., "Effect of Oral Immunization with Recombinant Urease on Murine Helicobacter felis Gastritis," Infection and Immunity, Apr. 1995, pp. 1246-1252.
Maurice M. Exner et al., "Isolation and characterization of a Family of Porin Proteins from Helicobacter pyroli , " Infection and Immunity, Apr. 1995, pp. 1567-1572.
Allan D. Pronovost et al., "Evaluation of a New Immunodiagnostic Assay for Helicobacter pylori Antibody Detection: Correlation with Histopathological and Microbiological Results," Journal of Clinical Microbiology, vol. 32, No. 1, Jan. 1994, pp. 46-50.
Steven J. Czinn et al., "Protection of germ--free mice from infection by Helicobacter felis after active oral of passive IgA immunization.", Vaccine, vol. 11, Issue 6, pp. 637-642, 1993.
C. Stewart Goodwin, Overview of Helicobacter Pylori Gastritis, Peptic Ulcer, and Gastric Cancer and the Possible Development of an H. Pylori Vaccine, Helicobacter pylori Biology and Clinical Practice, Chapter 25 pp. 431-444, 1993.

ART-UNIT: 165

PRIMARY-EXAMINER: Navarro; Albert

ATTY-AGENT-FIRM: Finnegan, Henderson, Farabow, Garrett and Dunner

ABSTRACT:

There is provided an immunogenic composition capable of inducing protective antibodies against Helicobacter infection characterized in that it comprises:

i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori (SEQ ID NO: 22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter felis urease (SEQ ID NO: 20-21), and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis (SEQ ID NO: 20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter pylori urease (SEQ ID NO: 22-26);

ii) and/or, a heat shock protein (Hsp), or chaperonin, from Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

20 Claims, 51 Drawing figures

US006258359B1

(12) **United States Patent**
Labigne et al.

(10) **Patent No.: US 6,258,359 B1**
(45) **Date of Patent: Jul. 10, 2001**

(54) **IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES**

(75) Inventors: **Agnes Labigne**, Bures sur Yvette (FR); **Sebastian Suerbaum**, Veitshöchheim (DE); **Richard L. Ferrero**, Paris; **Jean-Michel Thiberge**, Plaisir, both of (FR)

(73) Assignee: **Institut Pasteur**, Paris (FR)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/466,248**

(22) Filed: **Jun. 6, 1995**

Related U.S. Application Data

(60) Division of application No. 08/447,177, filed on May 19, 1995, now abandoned, which is a continuation-in-part of application No. 08/432,697, filed on May 2, 1995, which is a continuation-in-part of application No. PCT/EP94/01625, filed on May 19, 1994.

(30) **Foreign Application Priority Data**

May 19, 1993 (EP) 93 401 309
Nov. 19, 1993 (WO) PCT/EP93/03259

(51) **Int. Cl.**[7] **A61K 39/395**; A61K 39/40; C07K 1/00; C07K 16/00

(52) **U.S. Cl.** **424/141.1**; 424/150.1; 424/163.1; 424/164.1; 530/350; 530/388.1; 530/388.2; 530/388.4

(58) **Field of Search** 424/234.1, 141.1, 424/150.1, 163.1, 164.1; 514/2; 530/350, 388.1, 388.2, 388.4

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,538,729	*	7/1996	Czinn et al. .
5,858,773	*	1/1999	Mazodier et al. .
5,972,336	*	10/1999	Michetti et al. .

FOREIGN PATENT DOCUMENTS

WO 89/12455	* 12/1989	(WO) .
WOA9004030	4/1990	(WO) .
WOA9109049	6/1991	(WO) .
WOA9307273	4/1993	(WO) .
WOA9316723	9/1993	(WO) .
WOA9318150	9/1993	(WO) .
WOA9320843	10/1993	(WO) .
WOA9406474	3/1994	(WO) .
WOA9409823	5/1994	(WO) .
WOA9503824	2/1995	(WO) .
WO 9638475	12/1996	(WO) .
WO 9640893	12/1996	(WO) .

OTHER PUBLICATIONS

Roitt et al (Immunology, Grower Medical Publishing, London p. 601), 1985.*
Lazar et al. Molecular +Cellular Biology vol. 8 No. 3 pp 1247–1252, Mar. 1988.*
Burgess et al. J. of Cell Biology vol. 111 pp 2129–2138, Nov. 1990.*
Salgaller et al. Cancer Immunol. Immunother vol 39 pp 105–116, 1994.*
Smoot et al. Infection +Immunity vol. 58 No. 6 pp 1992–1994, Jun. 1990.*
Hu et al Infection +(Immunity vol. 60, No. 7, pp 2657–2666), Jul. 1992.*
Burns et al Journal of Biological Chemistry vol. 267 (36) pp 25632–25635, Dec. 25, 1992.*
Michetti et al (Gastroenterology vol. 107 pp 1002–1011), Oct. 1994.*
Hu et al (Infection+Immunity vol. 58 No 4. pp 992–998), Apr. 1990*
Sevier et al (Clinical Chemistry vol. 27 No. 11, pp 1797–1806, 1981).*
R.L. Ferrero et al., "Molecular Evidence Demonstrating Significant Homology Between the Urease Polypeptides of Helicobacter Felis and and Helicobacter Pylori," *Gastroenterology* , vol. 104, No. 4, Apr. 1993, Elsevier, New York, U.S.; P. A699.
E.G. Fox, et al. "Comparison of Two New Immunodiagnostic Assays for Helicobacter Pylori with Established Clinical and Histopathologic Findings", Gastroenterology, vol. 100, No. 5, Part 2, p. A66, 1991.
B.E. Dunn et al., "Identification and Purification of a cpn60 Heat Shock Protein Homolog from Helicobacter Pylori, " *Infection and Immunity*, vol. 60, No. 5, May 1992, Am. Soc. Microbiol., Baltimore, US; pp. 1946–1951.
D.J. Evans et al., "Urease–associated Heat Shock Protein of Helicobacter Pylori," *Infection and Immunity*, vol. 60, No. 5, May 1992, Am, Soc. Microbiol., Baltimore, US; pp. 2125–2127.

(List continued on next page.)

Primary Examiner—Albert Navarro
(74) *Attorney, Agent, or Firm*—Finnegan, Henderson, Farabow, Garrett and Dunner

(57) **ABSTRACT**

There is provided an immunogenic composition capable of inducing protective antibodies against Helicobacter infection characterized in that it comprises:

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NO: 22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis urease* (SEQ ID NO: 20–21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NO: 20–21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori urease* (SEQ ID NO: 22–26);

ii) and/or, a heat shock protein (Hsp), or chaperonin, from Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

20 Claims, 36 Drawing Sheets

DOCUMENT-IDENTIFIER: US 6248330 B1
** See image for Certificate of Correction **
TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):
i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori, or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter felis urease, and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis, or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter pylori urease;

CLAIMS:

10. Proteinaceous material comprising a fusion protein, wherein the fusion protein comprises at least one Helicobacter HspA or a fragment thereof as defined in any one of claims 6-9 and at least one polypeptide selected from the group consisting of

a Helicobacter pylori urease structural polypeptide or fragment thereof, wherein said fragment is recognized by antibodies to H. felis urease, and

a Helicobacter felis urease structural polypeptide or immunogenic fragment thereof.

11. An immunogenic composition, which induces antibodies against Helicobacter infection, comprising at least one sub-unit of a purified, synthetic, or recombinant Helicobacter felis urease structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, and a heat shock protein (Hsp) from Helicobacter or a fragment thereof, wherein the Hsp protein is HspA or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), and wherein said fragment has at least 6 amino acids and is immunogenic.

16. An immunogenic composition, capable of inducing antibodies against Helicobacter infection, comprising at least one sub-unit of a purified, synthetic, or recombinant Helicobacter felis urease structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, further comprising at least one heat shock protein (Hsp) from Helicobacter, wherein the Hsp protein is HspA, HspB, or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), or a fragment thereof, wherein said fragment has at least 6 amino acids and is capable of generating antibodies.

US006248330B1

(12) **United States Patent**
Labigne et al.

(10) **Patent No.: US 6,248,330 B1**
(45) **Date of Patent: *Jun. 19, 2001**

(54) **IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES**

(75) Inventors: **Agnes Labigne**, Bures sur Yvette (FR); **Sebastien Suerbaum**, Bochum (DE); **Richard L. Ferrero**, Paris; **Jean-Michel Thiberge**, Plaisir, both of (FR)

(73) Assignee: **Institut Pasteur**, Paris (FR)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **08/432,697**

(22) Filed: **May 2, 1995**

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/EP94/01625, filed on May 19, 1994.

(30) **Foreign Application Priority Data**

May 19, 1993 (EP) 93401309
May 19, 1994 (WO) PCT/EP94/03259

(51) **Int. Cl.**[7] **A61K 39/00**
(52) **U.S. Cl.** **424/192.1**; 424/234.1; 424/184.1; 435/6; 435/69.1
(58) **Field of Search** 424/234.1, 184.1, 424/203.1, 192.1; 435/6, 7.21

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,262,156 * 11/1993 Alemohammad 424/92
5,459,041 * 10/1995 Blaser et al. 435/7.21
5,538,729 * 7/1996 Czinn et al. 424/234.1

FOREIGN PATENT DOCUMENTS

9004030 * 4/1990 (WO) .
WOA9004030 4/1990 (WO) .
WOA9109049 6/1991 (WO) .
9109049 * 6/1991 (WO) .
WOA9307273 4/1993 (WO) .
WOA9316723 9/1993 (WO) .
WOA9318150 9/1993 (WO) .
WOA9320843 10/1993 (WO) .
WOA9406474 3/1994 (WO) .

(List continued on next page.)

OTHER PUBLICATIONS

Labigne, A et al, Am. Gastroenterol. Hepatol. (France) Mar.–Apr. 1992, 28(2) pp. 93–97 (abstract only in English).*

(List continued on next page.)

Primary Examiner—Christopher L. Chin
Assistant Examiner—Ginny Allen Portner
(74) *Attorney, Agent, or Firm*—Finnegan, Henderson, Farabow, Garrett & Dunner

(57) **ABSTRACT**

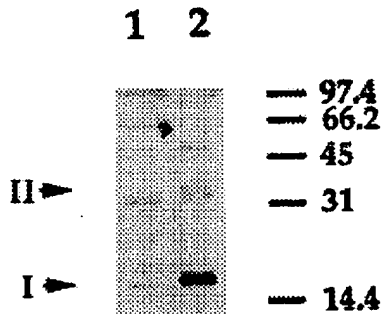
There is provided an immunogenic composition capable of inducing protective antibodies against Helicobacter infection characterized in that it comprises:

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease, and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease;

ii) and/or, a heat shock protein (Hsp), or chaperonin, from Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

16 Claims, 36 Drawing Sheets



DOCUMENT-IDENTIFIER: US 5843460 A
TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):
i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter felis urease (SEQ ID NOS:20-21), and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis (SEQ ID NOS:20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter pylori urease (SEQ ID NOS:22-26);

CLAIMS:

1. An immunogenic composition, capable of inducing antibodies against Helicobacter infection, comprising:

i) at least one urease structural polypeptide encoded by the UreB gene of Helicobacter pylori or Helicobacter felis or immunogenic fragment thereof comprising at least six consecutive amino acids; and

ii) at least one heat shock protein encoded by the Hsp A gene of Helicobacter pylori or Helicobacter felis or immunogenic fragment thereof, comprising at least 6 consecutive amino acids,

said composition being substantially free of other Helicobacter pylori or Helicobacter felis proteins.

2. An immunogenic composition comprising an immunizing amount of a mixture of Helicobacter pylori or Helicobacter felis antigens, wherein said mixture consists essentially of UreB and HspA of H. pylori or H. felis substantially free of other H. pylori or H felis proteins.

6. The immunogenic composition according to claim 1 or claim 2, wherein said composition produces an immunogenic effect when administered to a mammal, wherein the immunogenic effect is substantially the same as the immunogenic effect produced in the mammal when a total cell extract of Helicobacter pylori or Helicobacter felis is administered to said mammal.

10. A pharmaceutical composition for use in a vaccine against Helicobacter pylori or Helicobacter felis, comprising the immunogenic composition according to claim 1 or claim 2, in combination with a pharmaceutically acceptable carrier.

US005843460A

United States Patent [19]

Labigne et al.

[11] Patent Number: 5,843,460

[45] Date of Patent: Dec. 1, 1998

[54] **IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES**

[75] Inventors: **Agnes Labigne**, Bures S/Yvette, France; **Sebastin Suerbaum**, Bochum, Germany; **Richard L. Ferrero**, Paris; **Jean-Michel Thiberge**, Plaisir, both of France

[73] Assignees: **Institut Pasteur**; **Institut National de la Sante et de la Recherche Medicale**, both of Paris, France

[21] Appl. No.: **467,822**

[22] Filed: **Jun. 6, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 447,177, May 19, 1995, which is a continuation-in-part of Ser. No. 432,697, May 2, 1995.

[30] **Foreign Application Priority Data**

May 19, 1993 [EP] European Pat. Off. 93 401 309
Nov. 19, 1993 [WO] WIPO PCT/EP93/03259

[51] **Int. Cl.**[6] **A61K 39/02**

[52] **U.S. Cl.** **424/234.1**; 435/7.32; 435/6; 435/7.9; 514/234.5; 514/41

[58] **Field of Search** 435/7.32, 4, 6, 435/7.9; 514/234.5, 41; 424/234.1

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

WOA9004030	4/1990	WIPO .
WOA9109049	6/1991	WIPO .
WOA9307273	4/1993	WIPO .
WOA9316723	9/1993	WIPO .
WOA9318150	9/1993	WIPO .
WOA9320843	10/1993	WIPO .
WOA9406474	3/1994	WIPO .
WOA9409823	5/1994	WIPO .
WOA9503824	2/1995	WIPO .
WO 9638475	12/1996	WIPO .
WO 9640893	12/1996	WIPO .

OTHER PUBLICATIONS

R. L. Ferrero et al., "Molecular Evidence Demonstrating Significant Homology Between the Urease Polypeptides of Helicobacter Felis and and Helicobacter Pylori," *Gastroenterology*, vol. 104, No. 4, Apr. 1993, Elsevier, New York, U.S.; p. A699.

E.G. Fox, et al. "Comparison of Two New Immunodiagnostic Assays for Helicobacter Pylori with Established Clinical and Histopathologic Findings", Gastroeneterology, vol. 100, No. 5, Part 2, p. A66.

B.E. Dunn et al., "Identification and Purification of a cpn60 Heat Shock Protein Homolog from Helicobacter Pylori," *Infection and Immunity*, vol. 60, No. 5, May 1992, Am. Soc. Microbiol., Baltimore, US; pp. 1946–1951.

(List continued on next page.)

Primary Examiner—James C. Housel
Assistant Examiner—Ginny Allen Portner
Attorney, Agent, or Firm—Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.

[57] **ABSTRACT**

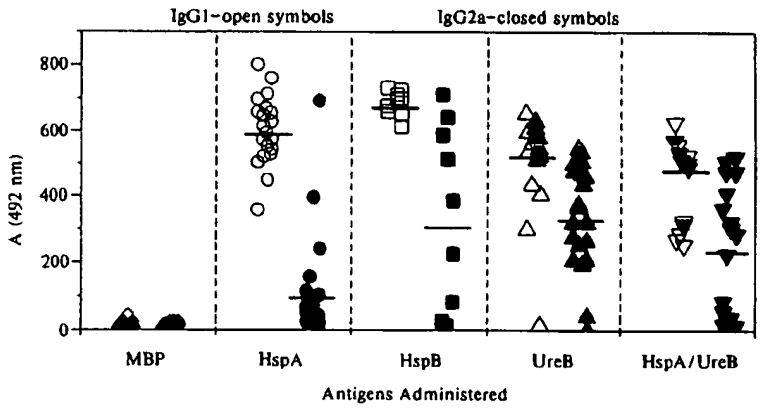
There is provided an immunogenic composition capable of inducing protective antibodies against Helicobacter infection characterized in that it comprises:

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NOS:20–21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NOS:20–21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NOS:22–26);

ii) and/or, a heat shock protein (Hsp), or chaperonin, from Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

10 Claims, 36 Drawing Sheets



PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 39/106, C07K 16/12, A61K 39/40 — A1

(11) International Publication Number: WO 96/34624

(43) International Publication Date: 7 November 1996 (07.11.96)

(21) International Application Number: PCT/EP96/01834

(22) International Filing Date: 2 May 1996 (02.05.96)

(30) Priority Data:

432,697	2 May 1995 (02.05.95)	US
447,177	19 May 1995 (19.05.95)	US

(71) Applicants *(for all designated States except US)*: INSTITUT PASTEUR [FR/FR]; 25-28, rue du Dr.-Roux, F-75724 Paris Cédex 15 (FR). INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cédex 13 (FR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: LABIGNE, Agnès [FR/FR]; 47, avenue Beauséjour, F-91440 Bures-sur-Yvette (FR). SUERBAUM, Sébastian [DE/DE]; Kanalstrasse 6, D-44787 Bochum (DE). FERRERO, Richard, L. [IT/FR]; 60, avenue des Gobelins, F-75013 Paris (FR). THIBERGE, Jean-Michel [FR/FR]; 15, rue de la Ferronnerie, F-78370 Plaisir (FR).

(74) Agents: GUTMANN, Ernest et al.; Ernest Gutmann-Yves Plasseraud S.A., 3, rue Chauveau-Lagarde, F-75008 Paris (FR).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

(57) Abstract

There is provided an immunogenic composition capabale of inducing protective antibodies against *Helicobacter* infection characterized in that it comprises: i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease, and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease; ii) and/or, a heat shock protein (Hsp), or chaperonin, from *Helicobacter*, or a fragment of said protein. The preparation, by recombinant means, of such immunogenic composition is also provided.

L2: Entry 21 of 29 File: EPAB Nov 7, 1996

DOCUMENT-IDENTIFIER: WO 9634624 A1
TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

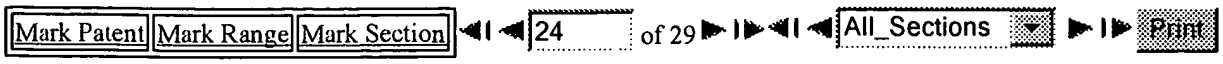
Abstract Text (1):
There is provided an immunogenic composition capabale of inducing protective antibodies against Helicobacter infection characterized in that it comprises: i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori, or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter felis urease, and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis, or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter pylori urease; ii) and/or, a heat shock protein (Hsp), or chaperonin, from Helicobacter, or a fragment of said protein. The preparation, by recombinant means, of such immunogenic composition is also provided.

DOCUMENT-IDENTIFIER: WO 9514093 A1
TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

Abstract Text (1):
CHG DATE=19990617 STATUS=O>The invention relates to an immunogenic composition, capable of inducing protective antibodies against Helicobacter infection, characterised in that it comprises: i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori, or a fragment thereof, said fragment being recognised by antibodies reacting with Helicobacter felis urease, and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis, or a fragment thereof, said fragment being recognised by antibodies reacting with Helicobacter pylori urease; ii) optionally, a urease-associated Heat Shock protein (HSP), or chaperonin, from Helicobacter, or a fragment of said protein.

DOCUMENT-IDENTIFIER: WO 9426901 A1
TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

Abstract Text (1):
CHG DATE=19990617 STATUS=O>The invention relates to an immunogenic composition, capable of inducing protective antibodies against Helicobacter infection, characterised in that it comprises: i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori, or a fragment thereof, said fragment being recognised by antibodies reacting with Helicobacter felis urease, and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis, or a fragment thereof, said fragment being recognised by antibodies reacting with Helicobacter pylori urease; ii) and/or, a Heat Shock protein (HSP), or chaperonin, from Helicobacter, or a fragment of said protein. The invention also relates to the preparation, by recombinant means, of such immunogenic compositions.


Mark Patent
Mark Range
Mark Section
24
of 29
All_Sections
Print

15. Document ID: US 6005090 A

L2: Entry 15 of 29 File: USPT Dec 21, 1999

DOCUMENT-IDENTIFIER: US 6005090 A
TITLE: Treatment and prevention of helicobacter infection

Abstract Text (1):
An antigenic preparation for use in the treatment or prevention of Helicobacter infection in a mammalian host, comprises the catalase enzyme of Helicobacter bacteria, particularly the catalase enzyme of H. pylori or H. felis, or an immunogenic fragment thereof.

CLAIMS:

8. A vaccine composition for use in the treatment or prevention of Helicobacter infection in a mammalian host, consisting essentially of an immunologically effective amount of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

9. A vaccine composition for use in the treatment or prevention of Helicobacter infection in a mammalian host, consisting essentially of an immunologically effective amount of Helicobacter pylori catalase, together with Helicobacter urease and Helicobacter lipopolysaccharide, and further together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

10. A method for the treatment or prevention of Helicobacter infection in a mammalian host, which comprises administration to said host of an immunologically effective amount of an antigenic preparation consisting essentially of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

11. A method for the treatment or prevention of Helicobacter infection in a mammalian host, which comprises administration to said host of an immunologically effective amount of an antigenic preparation consisting essentially of Helicobacter pylori catalase, together with Helicobacter urease and Helicobacter lipopolysaccharide, and further together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

12. A method of producing a vaccine, comprising the step of bringing an antigenic preparation into a form suitable for administration to a mammal, wherein said preparation consists essentially of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, and wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

13. Method of producing a vaccine, comprising the step of bringing an antigenic preparation into a form suitable for administration to a mammal, wherein said preparation consists essentially of Helicobacter pylori catalase, and Helicobacter urease and Helicobacter lipopolysaccharide, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

16. Document ID: US 5871749 A

L2: Entry 16 of 29 File: USPT Feb 16, 1999

DOCUMENT-IDENTIFIER: US 5871749 A
TITLE: Therapeutic treatment of H. pylori associated gastroduodenal disease

CLAIMS:

3. A method according to claim 1, wherein said one or more Helicobacter antigens comprise one or more H. felis antigens.

12. A method according to claim 10, wherein said one or more Helicobacter antigens comprise one or more H. felis antigens.

19. A method according to claim 11, wherein said one or more Helicobacter antigens is selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

20. A method according to claim 10, wherein said one or more Helicobacter antigens is selected from the group consisting of H. pylori urease, H. pylori cytotoxin, H. pylori cytotoxin associated immunodominant antigen, and H. pylori heat shock protein.

17. Document ID: US 5843460 A

L2: Entry 17 of 29 File: USPT Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843460 A
TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):
i) at least one sub-unit of a urease structural polypeptide from Helicobacter pylori (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter felis urease (SEQ ID NOS:20-21), and/or at least one sub-unit of a urease structural polypeptide from Helicobacter felis (SEQ ID NOS:20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with Helicobacter pylori urease (SEQ ID NOS:22-26);

CLAIMS:

1. An immunogenic composition, capable of inducing antibodies against Helicobacter infection, comprising:

i) at least one urease structural polypeptide encoded by the UreB gene of Helicobacter pylori or Helicobacter felis or immunogenic fragment thereof

comprising at least six consecutive amino acids; and

ii) at least one heat shock protein encoded by the Hsp A gene of Helicobacter pylori or Helicobacter felis or immunogenic fragment thereof, comprising at least 6 consecutive amino acids,

said composition being substantially free of other Helicobacter pylori or Helicobacter felis proteins.

2. An immunogenic composition comprising an immunizing amount of a mixture of Helicobacter pylori or Helicobacter felis antigens, wherein said mixture consists essentially of UreB and HspA of H. pylori or H. felis substantially free of other H. pylori or H felis proteins.

6. The immunogenic composition according to claim 1 or claim 2, wherein said composition produces an immunogenic effect when administered to a mammal, wherein the immunogenic effect is substantially the same as the immunogenic effect produced in the mammal when a total cell extract of Helicobacter pylori or Helicobacter felis is administered to said mammal.

10. A pharmaceutical composition for use in a vaccine against Helicobacter pylori or Helicobacter felis, comprising the immunogenic composition according to claim 1 or claim 2, in combination with a pharmaceutical carrier.

☐ 18. Document ID: JP 2004337170 A

L2: Entry 18 of 29 File: JPAB Dec 2, 2004

PUB-NO: JP02004337170A
DOCUMENT-IDENTIFIER: JP 2004337170 A
TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

PUBN-DATE: December 2, 2004

INVENTOR-INFORMATION:

NAME	COUNTRY
LABIGNE, AGNES	
SUERBAUM, SEBASTIEN	
FERRERO, RICHARD	
THIBERGE, JEAN-MICHEL	

INT-CL (IPC): C12 N 15/09; A61 K 38/00; A61 K 39/00; A61 K 39/39; A61 K 39/395; A61 P 1/04; A61 P 35/00; A61 P 37/02; C07 K 14/205; C07 K 16/12; C07 K 16/40; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/80; C12 Q 1/68; C12 P 21/08

☐ 19. Document ID: JP 2002355054 A

L2: Entry 19 of 29 File: JPAB Dec 10, 2002

PUB-NO: JP02002355054A
DOCUMENT-IDENTIFIER: JP 2002355054 A
TITLE: HELICOBACTER FELIS VACCINE

PUBN-DATE: December 10, 2002

INVENTOR-INFORMATION:

NAME	COUNTRY
KUSTERS, JOHANNES GERARDUS	
CATTOLI, GIOVANNI	

INT-CL (IPC): C12 N 15/09; A61 K 38/00; A61 K 39/106; A61 K 39/118; A61 K 39/12; A61 K 39/175; A61 K 39/23; A61 K 39/235 ; A61 K 39/39; A61 K 39/395; A61 P 1/04; A61 P 31/04; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/80; C12 Q 1/68; G01 N 33/15; G01 N 33/50; G01 N 33/53; G01 N 33/566; G01 N 33/569

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 20. Document ID: EP 1176192 A2

L2: Entry 20 of 29 File: EPAB Jan 30, 2002

PUB-NO: EP001176192A2
DOCUMENT-IDENTIFIER: EP 1176192 A2
TITLE: Helicobacter felis vaccine

PUBN-DATE: January 30, 2002

INVENTOR-INFORMATION:

NAME	COUNTRY
KUSTERS, JOHANNES GERARDUS	NL
CATTOLI, GIOVANNI	IT

INT-CL (IPC): C12 N 9/80; C07 K 14/205; C07 K 16/12; C12 Q 1/68; A61 K 39/106; A61 K 48/00; G01 N 33/53; G01 N 33/68
EUR-CL (EPC): C07K014/205; C12N009/80

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 21. Document ID: WO 9634624 A1

L2: Entry 21 of 29 File: EPAB Nov 7, 1996

PUB-NO: WO009634624A1
DOCUMENT-IDENTIFIER: WO 9634624 A1
TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

PUBN-DATE: November 7, 1996

INVENTOR-INFORMATION:

NAME	COUNTRY
LABIGNE, AGNES	FR
SUERBAUM, SEBASTIAN	DE
FERRERO, RICHARD L	FR
THIBERGE, JEAN-MICHEL	FR

INT-CL (IPC): A61 K 39/106; C07 K 16/12; A61 K 39/40
EUR-CL (EPC): A61K039/106; C07K014/205, C07K016/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw De

---

☐ 22. Document ID: WO 9514093 A1

L2: Entry 22 of 29 File: EPAB May 26, 1995

PUB-NO: WO009514093A1
DOCUMENT-IDENTIFIER: WO 9514093 A1
TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

PUBN-DATE: May 26, 1995

INVENTOR-INFORMATION:

NAME	COUNTRY
LABIGNE, AGNES	
SUERBAUM, SEBASTIEN	
FERRERO, RICHARD	

INT-CL (IPC): C12 N 15/31; C12 N 9/80; C12 Q 1/68; C12 P 21/08; A61 K 39/106
EUR-CL (EPC): C07K014/205; C12N009/80, C12Q001/68

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw De

---

☐ 23. Document ID: WO 9426901 A1

L2: Entry 23 of 29 File: EPAB Nov 24, 1994

PUB-NO: WO009426901A1
DOCUMENT-IDENTIFIER: WO 9426901 A1
TITLE: IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES

PUBN-DATE: November 24, 1994

INVENTOR-INFORMATION:

NAME	COUNTRY
LABIGNE, AGNES	FR

SUERBAUM, SEBASTIEN FR
FERRERO, RICHARD FR
THIBERGE, JEAN-MICHEL FR

INT-CL (IPC): C12N 15/31; C12N 9/80; C12Q 1/68; C12P 21/08; A61K 39/106; G01N 33/577
EUR-CL (EPC): C07K014/205; C12N009/80, C12Q001/68

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw De

☐ 24. Document ID: ES 2208519 T3, EP 1176192 A2, AU 200154321 A, CA 2351110 A1, JP 2002355054 A, EP 1176192 B1, DE 60100879 E, US 20040005325 A1

L2: Entry 24 of 29 File: DWPI Jun 16, 2004

DERWENT-ACC-NO: 2002-124384
DERWENT-WEEK: 200442
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Novel Helicobacter felis urease X and Y subunit polypeptides, useful in the diagnosis of Helicobacter felis infections and in the preparation of vaccines

INVENTOR: CATTOLI, G; KUSTERS, J G

PRIORITY-DATA: 2000EP-0202565 (July 17, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
ES 2208519 T3	June 16, 2004		000	C12N009/80
EP 1176192 A2	January 30, 2002	E	076	C12N009/80
AU 200154321 A	January 24, 2002		000	C12N015/52
CA 2351110 A1	January 17, 2002	E	000	C12N015/55
JP 2002355054 A	December 10, 2002		149	C12N015/09
EP 1176192 B1	October 1, 2003	E	000	C12N009/80
DE 60100879 E	November 6, 2003		000	C12N009/80
US 20040005325 A1	January 8, 2004		000	A61K039/00

INT-CL (IPC): A61 K 38/00; A61 K 39/00; A61 K 39/106; A61 K 39/118; A61 K 39/12; A61 K 39/175; A61 K 39/23; A61 K 39/235 ; A61 K 39/38; A61 K 39/39; A61 K 39/395; A61 K 48/00; A61 P 1/04; A61 P 31/04; C07 K 14/195; C07 K 14/205 ; C07 K 16/12; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/80; C12 N 15/09; C12 N 15/52; C12 N 15/55; C12 Q 1/68; G01 N 33/15; G01 N 33/50; G01 N 33/53; G01 N 33/566; G01 N 33/569; G01 N 33/68; C12 N 9/80; C12 Q 1/68; C12 R 1:01; C12 R 1:01

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw De

☐ 25. Document ID: NZ 519980 A, WO 200152667 A2, AU 200140494 A, EP 1118271 A1, NO 200203434 A, BR 200016973 A, EP 1251747 A2, US 20030049240 A1, JP 2003520038 W, MX 2002007018 A1, ZA 200206546 A

L2: Entry 25 of 29 File: DWPI Apr 30, 2004

DERWENT-ACC-NO: 2002-280180
DERWENT-WEEK: 200431
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Use of lactic acid bacteria strain(s), metabolites or fermented medium, having anti-Helicobacter activity in vitro, in preparation of composition for prophylaxis/treatment of gastric disorders in pets

INVENTOR: BALLEVRE, O; CORTHESY-THEULAZ, I ; ENSLEN, M Y A ; ENSLEN, A M

PRIORITY-DATA: 2000EP-0200179 (January 18, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
NZ 519980 A	April 30, 2004		000	A23K001/16
WO 200152667 A2	July 26, 2001	E	027	A23K001/00
AU 200140494 A	July 31, 2001		000	A23K001/00
EP 1118271 A1	July 25, 2001	E	000	A23K001/16
NO 200203434 A	August 20, 2002		000	A23K000/00
BR 200016973 A	October 15, 2002		000	A23K001/00
EP 1251747 A2	October 30, 2002	E	000	A23K001/16
US 20030049240 A1	March 13, 2003		000	A61K045/00
JP 2003520038 W	July 2, 2003		033	A23K001/00
MX 2002007018 A1	January 1, 2003		000	A23K001/00
ZA 200206546 A	March 31, 2004		036	A23K000/00

INT-CL (IPC): A23 K 0/00; A23 K 1/00; A23 K 1/16; A23 K 1/18; A61 K 35/74; A61 K 45/00; A61 P 1/04; A61 P 31/04

☐ 26. Document ID: WO 200003730 A1, AU 9884643 A

L2: Entry 26 of 29 File: DWPI Jan 27, 2000

DERWENT-ACC-NO: 2000-171201
DERWENT-WEEK: 200029
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Use of Lactobacillus-derived urease, as a vaccine for preventing or treating Helicobacter infection and conditions including gastric ulcer, gastritis and gastric cancer caused by the infection

INVENTOR: PARK, J B

PRIORITY-DATA: 1998WO-KR00216 (July 16, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200003730 A1	January 27, 2000	E	038	A61K039/07
AU 9884643 A	February 7, 2000		000	A61K039/07

INT-CL (IPC): A61 K 39/07; C07 K 14/335

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 27. Document ID: WO 9911284 A1, AU 9888779 A

L2: Entry 27 of 29 File: DWPI Mar 11, 1999

DERWENT-ACC-NO: 1999-204981
DERWENT-WEEK: 199931
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Vaccine containing lactobacilli expressing urease peptide - for treating gastrointestinal disorders

INVENTOR: TABAQCHALI, S; WILKS, M

PRIORITY-DATA: 1997GB-0018616 (September 2, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9911284 A1	March 11, 1999	E	023	A61K039/02
AU 9888779 A	March 22, 1999		000	A61K039/02

INT-CL (IPC): A61 K 38/43; A61 K 39/02; C12 N 15/74

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 28. Document ID: WO 9634624 A1, AU 9656934 A

L2: Entry 28 of 29 File: DWPI Nov 7, 1996

DERWENT-ACC-NO: 1996-505900
DERWENT-WEEK: 199904
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New immunogenic compsn. contg. UreB and HspA antigens of Helicobacter - for treatment and prevention of esp. H pylori infection, also new antibodies specific for these antigens.

INVENTOR: FERRERO, R L; LABIGNE, A ; SUERBAUM, S ; THIBERGE, J

PRIORITY-DATA: 1995US-0447177 (May 19, 1995), 1995US-0432697 (May 19, 1995)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9634624 A1	November 7, 1996	E	184	A61K039/106
AU 9656934 A	November 21, 1996		000	A61K039/106

INT-CL (IPC): A61 K 39/106; A61 K 39/40; C07 K 16/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 29. Document ID: JP 2004337170 A, WO 9426901 A1, AU 9469290 A, EP 703981 A1, JP 08510120 W, AU 689779 B, AU 9875081 A, SG 52480 A1, US 5843460 A, AU 724584 B, US 6248330 B1, US 6258359 B1

L2: Entry 29 of 29 File: DWPI Dec 2, 2004

DERWENT-ACC-NO: 1995-006797
DERWENT-WEEK: 200479

TITLE: DNA from Helicobacter pylori and Helicobacter felis - used to develop prods. for detection, treatment and prevention of Helicobacter infection

INVENTOR: FERRERO, R; LABIGNE, A ; SUERBAUM, S ; THIBERGE, J ; FERRERO, R L

PRIORITY-DATA: 1993WO-EP03259 (November 19, 1993), 1993EP-0401309 (May 19, 1993), 1995JP-0514170 (November 19, 1993)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 2004337170 A	December 2, 2004		063	C12N015/09
WO 9426901 A1	November 24, 1994	E	168	C12N015/31
AU 9469290 A	December 12, 1994		000	C12N015/31
EP 703981 A1	April 3, 1996	E	000	C12N015/31
JP 08510120 W	October 29, 1996		154	C12N015/09
AU 689779 B	April 9, 1998		000	C12N015/31
AU 9875081 A	October 1, 1998		000	A61K039/106
SG 52480 A1	September 28, 1998		000	C12N015/31
US 5843460 A	December 1, 1998		000	A61K039/02
AU 724584 B	September 28, 2000		000	A61K039/106
US 6248330 B1	June 19, 2001		000	A61K039/00
US 6258359 B1	July 10, 2001		000	A61K039/395

INT-CL (IPC): A61 K 38/00; A61 K 39/00; A61 K 39/02; A61 K 39/106; A61 K 39/39; A61 K 39/395; A61 K 39/40; A61 P 1/04; A61 P 35/00; A61 P 37/02; C07 H 21/04; C07 K 1/00; C07 K 14/195; C07 K 14/205; C07 K 16/00; C07 K 16/12; C07 K 16/40; C12 N 1/15; C12 N 1/19; C12 N 1/21; C12 N 5/10; C12 N 9/80; C12 N 15/09; C12 N 15/31; C12 P 21/02; C12 P 21/08; C12 Q 1/68; G01 N 33/569; G01 N 33/574; G01 N 33/577

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Terms	Documents
(felis and urease).ti,ab,clm.	29

Display Format: - Change Format

Previous Page Next Page Go to Doc#

196341

STIC-ILL

From: Portner, Ginny
To: STIC-ILL
Subject: FROM 1802
Date: Tuesday, October 28, 1997 3:59PM

DEVELOPMENT OF AN ANIMAL MODEL TO TEST A HELICOBACTER-PYLORI VACCINE
DUNKLEY M L; CRIPPS A W; REINBOTT P W
AUSPHARM INST. MUCOSAL IMMUNOL., P.O. BOX 151, JESMOND, N.S.W. 2299, AUST.
VITH INTERNATIONAL WORKSHOP ON CAMPYLOBACTER HELICOBACTER AND RELATED ORGANISMS, SYDNEY, NEW SOUTH WALES, AUSTRALIA, OCTOBER 7-10, 1991. MICROB ECOL HEALTH DIS 4 (SPEC. ISSUE). 1991. S148. CODEN: MEHDE
Language: ENGLISH
Document Type: CONFERENCE PAPER
Descriptors/Keywords: ABSTRACT RAT GUT FREUND'S COMPLETE ADJUVANT IMMUNOGLOBULIN A IMMUNOGLOBULIN G IMMUNE RESPONSE INTRAMUSCULAR ADMINISTRATION ORAL ADMINISTRATION METHOD

(d-29
NO

LC
10131
SMP
Completed
0
2
20

SOMED
SOCIETY FOR MICROBIAL ECOLOGY AND DISEASE

The VIth International Workshop on *Campylobacter helicobacter* and Related Organisms, Sydney, Australia

VOLUME 4 (S)

OCTOBER 1991

CONTENTS

H5-3

DEVELOPMENT OF AN ANIMAL MODEL TO TEST A *HELICOBACTER PYLORI* VACCINE

M.L. Dunkley, A.W. Cripps and P.W. Reinbott

Auspharm Institute for Mucosal Immunology
P.O. Box 151, Jesmond NSW 2299

An animal model is being developed to test a vaccine against *H. Pylori* and to investigate the mechanism of the immune response to *H. Pylori* in the gut.

Initially, rats were immunized using several different immunization regimes, viz. intramuscular (IM) immunization with lyophilized *H. Pylori* in Freunds' complete adjuvant (FCA), intra-Peyers patch (IPP) immunization with paraformaldehyde-killed *H. Pylori* in FCA and oral immunization with live *H. Pylori*, lyophilized *H. Pylori* or paraformaldehyde-treated *H. Pylori*, all in phosphate buffered saline and with prior administration of sodium bicarbonate.

IM immunization produced a significant enhanced IgG and IgA *H. Pylori* specific antibody response in the serum but had no effect on the salivary antibody response. IPP immunization gave an enhanced serum and saliva IgG and IgA antibody response and the Peyers patch lymphocytes were demonstrated to have a substantial proliferative response to a crude *H. Pylori* antigen preparation in vitro (stimulation index=64 $\pm$ 26) indicating that the gut mucosa is capable of mounting a vigorous immune response against the *H. Pylori* bacteria. Oral immunization however, has been less successful. Oral immunization with live or para-formaldehyde-treated bacteria gave no significant enhancement of the serum or saliva anti-*H. Pylori* antibody. Oral immunization with lyophilized H. *Pylori* gave a small increase in serum antibody response but this was not significant. The enhancement of this response by the addition of adjuvants is under investigation.

MICROB ECOL HEALTH DIS 4: S119
nal Workshop on Campylobacter, Helicobacter
Related Organisms Oct 7-10, 1991

H2-4

IMMUNISATION AND GASTRIC COLONISATION WITH *HELICOBACTER FELIS*

Keith Heap and Adrian Lee

University of New South Wales, Sydney, Australia 2033

Introduction

There is accumulating evidence that long term infection with *Helicobacter pylori* is a prerequisite for the development of atrophic gastritis and the subsequent development of gastric cancer in a subset of persons in certain developing countries. Thus, introduction of intervention strategies at an early age may influence the morbidity and mortality of this serious disease. Immunisation would be an attractive option but, given *H. pylori* can survive in the body for tens of years in the presence of a strong immune response, may not be effective. *Helicobacter felis* will colonise the gastric mucosa of SPF mice in large numbers occupying the gastric pits and mucus. Like *H. pylori* in humans this bacterium will remain for the life of the animal. Thus, the *H. felis*- infected mouse would appear to be a good model to test the hypothesis that immunisation can protect against colonisation with gastric helicobacters.

Methods

SPF mice were immunised by intravenous injection of 0.1 ml of a suspension of viable *H. felis* (10^8 / ml) once a week for 5 weeks or infected *per os* over 5 days with three doses of the bacterium. Immune responses of both these groups of animals were measured. A similar group of parenterally immunised animals were challenged with living cultures of *H. felis*. A final group of orally *H. felis* -infected animals was cleared of the organism with triple anti-microbial therapy for 28 days (tetracycline, metronidazole, bismuth subcitrate). These animals and controls that had been given saline instead of triple therapy were then challenged with a living culture of *H. felis*. All challenged animals were assessed for *H. felis* colonisation by rapid urease testing of gastric tissue and histology.

Results

Parenteral immunisation of mice with living cultures of *H. felis* induced a very high level of serum IgG, significant IgM and IgA could be detected in the bile. Serum responses post oral infection were much less and developed slowly. Hyperimmunisation of mice with an intravenous injection of a live culture of *H. felis* had no protective effect on gastric colonisation. In contrast, in mice cleared of infection with *H. felis* by administration of a one month treatment of antibiotics, some effect on rechallenge was seen. Colonisation was significantly delayed, with numbers of animals showing no urease reactivity for up to 10 days after rechallenge with an inoculum of *H. felis* that always gave 100% positivity in normal animals.

Conclusion

Parenteral immunisation with *H. felis* gave absolutely no protection against gastric colonisation. The same is likely to be true for *H. pylori*. However, preliminary experiments show that previous oral infection with living bacteria did appear to have some effect on reinfection. Further experiments are in progress to assess the value of oral immunisation against infection with gastric helicobacters.

Development of a vaccine against *Helicobacter pylori*: a short overview

Rino Rappuoli, Massimo Bugnoli, Paolo Ghiara, Antonello Covacci, Roberto Olivieri, Zhaoying Xiang and John L. Telford

Aim: Growing evidence that gastric and duodenal disease is caused by *Helicobacter pylori* infection suggests that this disease may be prevented by vaccination. We therefore assessed the possibilities for development of a vaccine.

Method: Survey of published studies.

Present state of development: Development of a vaccine requires identification of the factors responsible for bacterial virulence and disease induction and large-scale production and testing of potential vaccines in animal models. So far several factors involved in bacterial adhesion, colonization and virulence have been identified. Among these, the most promising candidates for vaccine development are the adhesins, the vacuolating cytotoxin and urease. Urease-based vaccines have shown some promising results in protecting mice against *H. felis* infection.

Proposals: The unique features of *H. pylori* infection and disease formation in man suggest that vaccines should be tested in models more relevant to humans, and that the vacuolating cytotoxin and the cytotoxin-associated gene A (*cagA*) should be seriously considered as vaccine candidates. This hypothesis is supported by the recent observation that only the subset of strains that produce the vacuolating cytotoxin and *cagA* are associated with disease.

European Journal of Gastroenterology & Hepatology 1993, 5 (suppl 2):S76–S78

Keywords: Vaccine, cytotoxin-associated gene, vacuolating cytotoxin, *Helicobacter pylori*.

Introduction

The strong correlation between *Helicobacter pylori* infection and the incidence of gastritis, peptic ulcer and gastric cancer has provided conclusive evidence that this infection is a major factor in the genesis of gastric diseases. Vaccination is the classical way of preventing infectious diseases and therefore it is important to study this possibility of controlling *H. pylori* infection.

The development of a vaccine needs four major steps: (1) identification of the factors required for virulence, (2) large-scale production and characterization of the virulence factors, (3) development of appropriate animal models to test the virulence and immunogenicity of the molecules identified, and (4) identification of the type of immunity able to prevent infection and disease.

Identification of virulence factors

Many virulence factors of *H. pylori* have been identified during the last 10 years. These include factors involved in adhesion of the bacteria to eukaryotic cells, toxins and associated proteins, flagella, urease and heat-shock proteins.

Adhesins

Several factors involved in bacterial adhesion have been characterized and some of them have been cloned. These include molecules with weights of 19 600, 20 000 and 63 000 [1–3] that are candidates for inclusion in experimental vaccines, in order to evaluate their ability to induce antibodies able to prevent the bacterial adhesion to the mucosal surface.

Vacuolating cytotoxin and the cytotoxin-associated protein (CagA)

The molecule that is believed to be a major factor in *H. pylori* pathogenesis is the vacuolating cytotoxin. This is a protein with a molecular weight of 95 000 that is found in bacterial culture supernatants and on the external surface of the bacteria; it induces vacuolation in Hela cells or other cell lines of epithelial origin. The cells affected by the toxin form large cytoplasmic vacuoles and die within a few days. Formation of the vacuoles can be inhibited by the antibiotic bafilomycin

From the Immunobiology Research Institute Siena, Siena, Italy.

Requests for reprints to: Dr Rino Rappuoli, Immunobiology Research Institute Siena, Via Fiorentina 1, 53100 Siena, Italy.

A1, a specific inhibitor of eukaryotic vacuolar ATPase, suggesting that vacuole formation requires direct or indirect activation of the vacuolar ATPase enzyme, which pumps protons into the lumen of the vacuole [4].

Recently, it has been shown that only a subset of *H. pylori* isolates are able to induce vacuole formation. These strains produce and secrete in the supernatant the active cytotoxin, a monomer with a molecular weight of 95 000 [5]. Extracts of cytotoxic strains induce the formation of vacuoles not only in Hela cells, but also in the stomach of experimental animals, suggesting that the cytotoxin is an active disease factor. Cytotoxic *H. pylori* strains also produce a surface-exposed protein of unknown function with a molecular weight of 128 000. This protein, cytotoxin-associated gene A (CagA), varies in size in different isolates [6,7]. The sequence of the protein is well conserved and the difference in size is due to the duplication of a segment of 102 base pairs which encodes for the most hydrophilic portion of the molecule. CagA is the most immunogenic antigen of *H. pylori* and the sera of infected subjects have high levels of antibodies against it. Analysis of sera has shown that 100% of patients with duodenal ulcers and 60% of patients with gastric ulcers have high levels of antibodies against CagA [8]. This observation strongly suggests that disease is caused only by the strains that produce the cytotoxin and CagA, and shows that CagA is a marker for cytotoxic, disease-associated strains. *H. pylori* isolates can therefore be classified into two major categories, the cytotoxin- and CagA-producing strains, which are virulent and induce disease, and the non-cytotoxic strains, which are not associated with disease.

Urease

The urease enzyme is produced by all strains of *H. pylori*, and helps the bacterium to neutralize the acidity of the stomach in order to survive these hostile conditions. The enzyme is also present in other species of *Helicobacter* and is well conserved in the different species. It has two subunits with molecular weights of 64 000 and 30 000 which are assembled in a high-molecular-weight molecule containing two ring-like structures, each composed of six monomers of one of the subunits [9].

Flagella

H. pylori produces polar sheathed flagella, which are essential for the bacterial mobility that allows penetration of the viscous mucous layer coating the gastric mucosa. The flagella consist of two subunits (FlaA and FlaB) with molecular weights of 51 000 and 53 000, respectively. The central core of the flagella is composed of polymerized FlaA subunits surrounded by a membrane-like structure containing proteins and lipopolysaccharide [10].

Heat-shock protein

A protein homologous to the Hsp60 family has been purified from water extracts of *H. pylori*. This antigen is highly homologous to the human homolog and induces antibodies that may react with the self molecule, suggesting a potential role in disease [11].

Production and purification of virulence factors

One of the limiting steps in studying the virulence of *H. pylori* is the difficulty of growing the bacterium on a large scale and of purifying large quantities of antigens. The bacterium grows very slowly under micro aerophilic conditions in the presence of serum, a medium full of exogenous proteins that makes antigen purification difficult. In order to overcome these problems we have developed a new serum-free medium that allows us to grow the bacterium in synthetic medium containing cyclodextrin [12]. We can then grow the bacterium in fermenters and collect large volumes of supernatant and cell paste. The availability of these materials has allowed, for the first time, large-scale purification of cytotoxin, CagA, urease and Hsp60. These molecules are now being tested *in vitro* and *in vivo* to help determine their contribution to pathogenicity and immunity.

Animal models and vaccine development

H. pylori is found only in primates such as man, macaques, cynomolgous monkeys and baboons. Therefore, these animals are the only relevant models. However, given the limited number of experiments that can be performed in monkeys and man, the high costs and the long time required for the experiments, alternative models are needed. For the moment, the most popular model is *H. felis*, a strain that is easy to work with because it infects mice. However, the results coming from these experiments must be interpreted cautiously because although *H. felis* produces urease, it does not produce the cytotoxin or the CagA antigen. We are at present investigating the possibility of infecting mice with virulent *H. pylori* strains and of mimicking the symptoms of the disease using purified *H. pylori* antigens.

Conclusion

The observation that most gastric pathologies are associated with *H. pylori* infection suggest that vaccination may be successful in preventing infection and disease. To test this hypothesis, several antigens have been identified as candidates for inclusion in experimental vaccines. These are the vacuolating cytotoxin,

CagA, urease, the flagella and the adhesins. These antigens are being tested in animal models.

References

1. Doig P, Austin JW, Kostrzynska M, Trust TJ: Production of a conserved adhesin by the human gastroduodenal pathogen *Helicobacter pylori*. *J Bacteriol* 1992, 174:2539–2547.
2. Evans DG, Karjalainen TK, Evans DJ, Graham DY, Lee C-H: Cloning, nucleotide sequence, and expression of a gene encoding an adhesin subunit protein of *Helicobacter pylori*. *J Bacteriol* 1993, 175:674–683.
3. Ingwood CL, Wasey G, Han H, Huesca M: Receptor affinity purification of a lipid-binding adhesin from *Helicobacter pylori*. *Infect Immun* 1993, 61:2474–2478.
4. Papini E, Bugnoli M, De Bernard M, Figura N, Rappuoli R, Montecucco C: Bafilomycin A1 inhibits *Helicobacter pylori* induced vacuolization of HeLa cells. *Mol Microbiol* 1992, 7:323–327.
5. Cover TL, Blaser MJ: Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. *J Biol Chem* 1992, 267:10570–10575.
6. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, *et al.*: Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993, 90:5791–5795.
7. Tummuru MKR, Cover TL, Blaser MJ: Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. *Infect Immun* 1993, 61:1799–1809.
8. Xiang Z, Bugnoli M, Rappuoli R, Covacci A, Ponzetto A, Crabtree JE. *Helicobacter pylori*: host response in peptic ulceration. *Lancet* 1993, 341:900–901.
9. Turbett GR, Hol PB, Horne R, Mee B: Purification and characterization of the urease enzymes of *Helicobacter* species from humans and animals. *Infect Immun* 1992, 60:3259–3266.
10. Levine II, Suerbaum S, Geis G, Haas R: Cloning and genetic characterization of a *Helicobacter pylori* flagellin gene. *Mol Microbiol* 1992, 6:2863–2874.
11. Macchia G, Massone A, Ruggiero D, Covacci A, Censini S, Rappuoli R: The hsp60 protein of *Helicobacter pylori*: structure and immune response in patients with gastroduodenal disease. *Mol Microbiol* in press.
12. Olivieri R, Bugnoli M, Armellini D, Bianciardi S, Rappuoli R, Bayeli PF, *et al.*: Growth of *Helicobacter pylori* in media containing cyclodextrins. *J Clin Microbiol* 1993, 31:160–162.

Best Copy

VVVI-VV72

HP WORLD-WIDE IS A QUARTERLY PUBLICATION GRANT FROM BROCADES PHARMA BV, LEIDERDORP, THE NETHERLANDS EDITORIAL ADDRESS. E
SCIENCE PUBLISHERS. P O. BOX 1527, 1000 BM AMSTERDAM, THE NETHERLANDS. HP WORLD-WIDE IS DISTRIBUTED ON REQUEST AND FREE OF CHA
GASTROENTEROLOGISTS AND PHYSICIANS WHO ARE INTERESTED IN GASTROENTEROLOGY COPYRIGHT· ELSEVIER SIENCE PUBLISHERS THE OPINIONS E
SED ARE NOT NECESSARILY THOSE OF THE PUBLISHERS OR SPONSOR.

HP WORLD-WIDE

H.pylori associated disease involves host and bacterial factors

Martin Blaser, Scoville Professor of Medicine and Professor of Microbiology and Immunology at the Vanderbilt University School of Medicine, Nashville, USA is a familiar name to those in the field of Campylobacter and Helicobacter research. He recently spoke to HPWW in Sydney and participated in a symposium at the European Digestive Disease Week in Amsterdam in October, speaking on pathogenic mechanisms involved in H.pylori infections.

"H pylori is a bacterium that the host cannot eliminate", said Blaser. "A critical factor is its ability to live in the lumen of the stomach without needing to invade tissue - it has succeeded where most gastrointestinal bacteria have failed. The host relies on acid, peristalsis, mucus and tight junctions to eliminate organisms from the stomach. H pylori not only avoids these host defences but initiates disease processes that can result in peptic ulceration and possibly gastric carcinoma."

Blaser proposed that the pathogenic mechanisms be divided into three groups: bacterial virulence factors, autoimmunity and what he terms 'the innocent bystander' theory.

Bacterial virulence factors

While a large number of putative virulence factors have been suggested, increasing attention is focused on the cytotoxin and the urease.

H.pylori is known to produce a toxin that causes cell vacuolation in vitro and this vacuolation is seen in vivo. "We have recently shown that people who are infected produce neutralising IgG antibodies against the cytotoxin - that means that the cytotoxin is produced in vivo", said Blaser.

Work by Blaser's group has shown that antibodies to a 128kDa cytotoxin-associated protein, are seen in 60% of patients with gastritis but in 100% of duodenal ulcer patients. This agrees with earlier work by Figura and colleagues who showed a higher frequency of cytotoxin-producing strains of H.pylori in ulcer patients. "There may be a group of H.pylori strains that put individuals at a higher risk of ulcer", suggested Blaser.

Dr Timothy Cover is currently purifying and characterising the cytotoxin. "Most people find that about 50-60% of strains express the cytotoxin. Once we purify the protein and clone the gene, we will know how many strains have the gene."

Prof. M Blaser

Blaser stressed that the cytotoxin and the urease are quite distinct. "It is clear to us that there is a urease effect and a cytotoxin effect and they are independent and additive in vitro. We have looked at urease-minus strains and they had cytotoxin and induced vacuolation. However ammonia augments the effect of the cytotoxin."

"The Helicobacters are the most potent producers of urease known", pointed out Blaser. Recent work has shown that at least nine genes are required for structure and regulation. "I'm impressed by the number of genes required for the regulation of urease. Obviously this activity is very important to the bacteria as it devotes so much metabolic energy to the production of urease. One of its functions is in acid buffering and facilitating colonisation - we are not yet certain what else it does."

The roles of other H.pylori enzymes such as the mucinase, oxidase, catalase, alkaline phosphatase and phospholipase are not yet clear. "Whether these enzymes are just performing housekeeping functions or are virulence factors for the bacterium, we don't know."

continued on page 2

in continuation of page 1

The innocent bystander

"The urease may have a role in pathogenesis quite unrelated to its enzymatic activity" suggested Blaser. The inflammation induced by H.pylori may be perturbing gastric physiology. How does a non-invasive organism cause inflammation?

"Urease is on the surface of H.pylori and there is evidence that it and other surface proteins can be shed from the organism and adsorbed into the lamina propria. We also know that, in vitro, H.pylori supernatants are chemotactic for both monocytes and neutrophils and this effect can be blocked by antibodies to the bacteria or to urease. An N-terminal peptide of the urease appears to be responsible for the chemotactic activity", said Blaser.

"These mononuclear cells are activated, producing superoxide and cytokines such as tissue necrosis factor and interleukin 1. Cytokines are known to stimulate gastrin production and the inflammation per se may be producing the hypergastrinemia seen in H.pylori infection", speculated Blaser.

This recruitment and activation of inflammatory cells may result in a positive feedback cycle, releasing nutrients for H.pylori. Blaser suggested that there are suppressor mechanisms that are trying to down-regulate this inflammation. "It is this interchange between suppression and inflammation that ultimately affects gastrin secretion and parietal cell function. This interchange may finally result in a diseased gastrointestinal tract, just as the innocent bystander may be caught in cross-fire between warring parties."

Autoimmunity and heat shock proteins

The Sydney meeting saw the report of a close homology between a surface-exposed, immunodominant H.pylori protein and a human heat-shock protein. All organisms from bacteria to mammalian cells have heat shock proteins, which are normal house-keeping proteins that respond to stress. These proteins are very highly conserved, both structurally and functionally.

"The heat shock protein that we identified with Bruce Dunn is in the chaperonin class of heat shock proteins - chaperonins are involved in the folding and transport of proteins. From our limited sequence data, the heat shock protein resembles the human heat shock protein, hsp 60, expressed on epithelial cells. We know that there is antigenic cross-reaction between H.pylori and a lot of other bacterial heat shock proteins which have been shown to cross-react with hsp 60. We haven't done the direct experiment yet but it is likely that it will cross-react."

Earlier evidence that autoimmunity might play a role came from work by B Rathbone who showed that a monoclonal antibody raised against H.pylori reacted with healthy gastric cells.

What determines outcome?

"We know that different people have different outcomes after infection. Most people have asymptomatic infection, some people develop duodenal ulcer, others gastric ulcer, others atrophic gastritis. The fundamental question is what is the determinant of each of those outcomes", stressed Blaser.

The age at which infection is acquired is almost certain to be one of the determinants, he agreed. In addition, there may be carcinogenic strains or ulcerogenic strains of H.pylori. "The outcome is most likely to be due to a combination of bacterial factors and host factors."

H.pylori affects both the antrum where gastrin is produced and the body where acid is produced. According to Blaser, "There must be a functional balance between the antrum and the body - in some people, the balance is disturbed in one direction or the other. I think it will be very important to characterise this phenomenon because that might determine who gets ulcers and who doesn't."

Is H.pylori a 'slow bacterium'?

"H.pylori might be the first of a group of organisms considered as 'slow bacteria' suggested Blaser, drawing an analogy with the so-called slow viruses, such as those responsible for scrapie in sheep and Creutzfeldt-Jacob disease in man. These agents are characterised by a long period of infection with chronic progressive pathology.

"This is a chronic infection as distinct from most bacterial infections which only last a few weeks. The bacteria are active and the host is aware of it, unlike infections such as tuberculosis or chickenpox which become latent and then reactivate. Pathogenicity should be measured not in days but in years." Prof Blaser drew attention to the limitations of experimental models of disease. "Something may be a real virulence factor and cause damage over the course of the infection, that is over decades, but the level of the damage is very small so you can't appreciate it in a model that lasts for a couple of days."

"In pathogenesis you always have to remember that there are in vivo events and in vitro events and the two are not necessarily related", cautioned Blaser. "What you see in vitro is not necessarily significant."

6th International Workshop on Campylobacter, Helicobacter and related organisms, October 7-10, 1991, Sydney

More and more Helicobacters

The first Helicobacter (then called Campylobacter pyloridis) was described at the second Campylobacter workshop in Brussels in 1983. These early days were remembered with a presentation to Robin Warren (Australia), the first to notice H.pylori in gastric biopsies. "Twelve years ago, no-one would believe me that the organisms were there," he said.

The number of believers has since steadily increased and many of them attended presentations and workshops on Helicobacter pathogenesis, drug resistance, therapy, immunology, epidemiology, animal models, taxonomy and genetics, in Sydney recently.

The number of Helicobacter species has now reached eight with descriptions at this meeting of H.acinonyx from cheetahs with gastritis (KA Eaton, USA), H.muridarum from the intestinal mucosa of the rodent (A Lee, Australia) and Helicobacter strains from the faeces of terns, gulls, house sparrows and pigs (J Fox, USA).

It appears that what was initially thought to be a homogeneous group confined to the stomach is heterogeneous in terms of habitat, ultrastructure and physiology. Some of these species are urease-negative so urease-positivity can no longer be considered a valid taxonomic marker. The only shared feature seems to be the presence of sheathed flagella. This heterogeneity has stimulated study of the genetics of Helicobacters and the development of genus- and species-specific probes.

Highly variable genome

The Helicobacter genomes are small, extremely variable and subject to natural transformation. Understanding the genetics allows the construction of specific mutants that should help clarify the role of various H.pylori products.

Many of the genetic studies have focused on the urease, which has been shown to have two structural genes, two regulatory genes and five other genes (A Labigne, France).

R Ferrero (France) reported the construction of stable genetically engineered urease mutants of H.pylori, affected either in the structural or the regulatory genes. Urease mutants were also constructed by L Tompkins and coworkers (USA) who reported mutants with inactive or temperature sensitive ureases or with very low urease activity.

Molecular biology can also be a valuable epidemiological tool. Examination of the restriction enzyme digest patterns of the ribosomal RNA genes revealed 77 different ribotypes from 100 patients (W Tee, Australia). Sequential isolates from the same patient revealed identical patterns confirming that infection with a particular strain persists. Such a method provides a highly discriminatory typing scheme although it is unsuitable for routine laboratories.

Molecular biology can also aid in detection of very small numbers of organisms as demonstrated by TU Westblom (USA) who detected H.pylori in 1/5 saliva samples from H.pylori-infected patients using the polymerase chain reaction.

What does the urease do?

The urease is likely to be important in colonisation, although urease-negative mutants do colonise, even though infection is delayed. It may also be an important source of nutrients.

Positive correlations between the numbers of H.pylori and the extent of gastritis, and between gastritis and the level of ammonia in the gastric juice, lend support to the idea of ammonia as a pathogenic factor (S Takahashi, Japan).

Studies reported with a urease inhibitor are discouraging for those who see the urease as a therapeutic target. Fluoramide, the most potent specific urease inhibitor yet described, abolishes the in vitro tolerance of H.pylori to acid in the presence of physiological concentrations of urea (AA McColm, UK). However in vivo studies in ferrets and monkeys show no effect on the organism despite considerable urease inhibition. The authors suggested that the inhibitor might only be effective during initial colonisation during bacterial transit across the lumen.

One of the most interesting reports concerned the identification of an H.pylori protein homologous with the human heat shock protein, hsp 60 (B Dunn, USA). This

major surface-exposed protein in H pylori is highly immunogenic (B Dunn, USA). The authors suggested that the H pylori protein contributes to gastric injury by stimulating T cells which cross-react with similar determinants present on endogenous heat shock proteins from stressed host cells.

H pylori produces at least two classes of adhesins, a shared adhesin and hemagglutinins of different specificities (P Doig, Canada). The authors suggested that these adhesins offer goals for therapy by: immunisation to provoke an immune response to H.pylori adhesins; competitive inhibition with synthetic adhesin peptides; adhesin receptor megatherapy.

An interesting study that may give some clues to the factors influencing colonisation was presented by N Coltro (Australia). Although H.muridarum is normally found in the ileum of infected mice, changes in the gastric environment due to colonisation with H.felis or G.hominis allow it to colonise the gastric mucosa.

Immunisation not promising

Many new serological kits were evaluated, including second-generation kits using combinations of purified antigens. It was stressed that serological assays need to be standardised and validated in each population.

While the immune response may help the clinician in diagnosis or in monitoring therapy, it does not appear to greatly benefit the host. The immune response does not limit infection (although no systemic infection occurs) but may prevent reinfection. However higher IgA levels were observed in patients with less inflammation, suggesting that specific IgA could be in some degree protective against tissue injury in H.pylori infection (GI Perez-Perez, USA).

Immunisation does not appear promising. Parenteral immunisation of specific pathogen free mice with H.felis gave no protection against gastric colonisation; previous oral infection only delayed colonisation (K Heap, Australia). Although intra-Peyers' patch immunisation of killed H.pylori in rats shows that the gut mucosa can mount a vigorous immune response, oral immunisation with either live or killed bacteria induced no significant serum or saliva antibody response (M Dunkley, Australia).

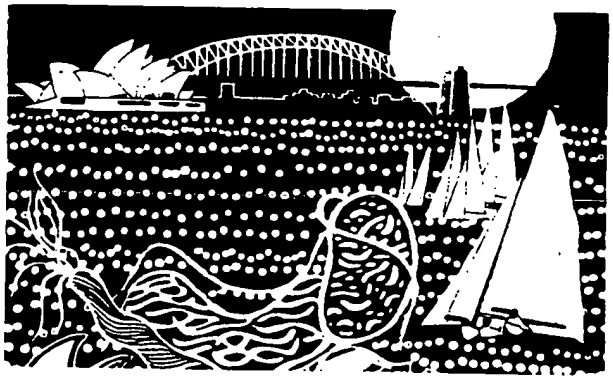
M Blaser (USA) warned that because of the possible autoimmune component of the disease the wrong vaccine could actually make things worse.

Helicobacter mustelae isolated from faeces

Further evidence for transmission within the family came from H Mitchell (Australia). Serial serum samples were taken from children from a low-income area in Mexico City. By the age of 2, 50% of children had H.pylori antibodies with the highest frequency of seroconversion between 5 and 15 months.

The faecal-oral theory received support from data from J Fox's group (USA) showing isolation of H.mustelae and other Helicobacter species from the faeces of 24/26 9-10 week old ferrets. Negative faecal cultures from the same animals at 20 weeks suggested that organisms were only shed during the period of transient hypochlorhydria following natural infection. However H.mustelae were also isolated from 3/4 8 month old ferrets. These observations suggest a number of possibilities. ferrets with acidic pH can shed the organism; H.mustelae-associated hypochlorhydria can exist for longer periods of time in selected ferrets or H.mustelae is shed in the faeces intermittently.

Dr. R. Warren (left) receives plaque from Prof. A. Lee in recognition of his role in the discovery of H. pylori."



Earlier suggestions that water supply was a source of infection were not confirmed by H Mitchell (Australia) who found from studies in rural and urban regions in Southern China that water source was not a significant factor in H pylori infection. Infection was significantly higher in urban (53%) than in rural (38%) populations, suggesting increased probability of person to person spread due to high city population density.

H felis survived in mucosal scrapings for 5 days at 22 degrees and 2 weeks at 40 degrees without losing infectivity (KS Diker, Turkey).

Australian gastroenterologists are obviously nervous about H pylori, and rightly so it seems. A second study on H pylori infection (M Schembri, Australia) in this group showed the prevalence of H pylori infection to be significantly higher than in general physicians, gastroenterology nurses and a control population. Prevalence increased with years of practice with 83% of gastroenterologists who had practised for more than 10 years having H pylori antibodies!

Antibiotic concentration at mucosa important

The main issues addressed [in] therapy included in vitro sensitivity [to] the role of acid suppression in [H] eradication and the pharmacology [of] biotics and bismuth compounds [in] tarty mucosal delivery and new [drug] therapy.

Y Glupczynski (Belgium) pointed [out] in vitro testing should only be used [as] first screen - resistance probably pred[icts] poor clinical outcome. In vitro [results] do not reflect the drug concentration [at] mucosa nor local conditions at the [site] infection.

A number of those present felt contrary to the Working Party's conservative recommendations to only treat [duodenal] ulcer patients with complication or frequent relapse, all duodenal ulcer patients should be treated. Once the complications have occurred it is too late.

The trend to shorter treatment regimens was illustrated by a report of 47% eradication after 4 days of De-Nol, tetracycline, tinidazole (Y Glupczynski, Belgium).

The importance of the local interactions of antibiotics was addressed. [Some] antagonists may increase mucosal concentrations. Omeprazole while effective in eradicating H pylori may also enhance antibiotic efficacy by increasing the local concentration and activity.

W Bar (Germany) showed that bismuth compounds reduced electron flow, indicating that the respiratory chain is the target.

What are the future options for therapy? Current triple therapy may be improved by pH control and manipulating dosages and formulations. There is a need to understand the pharmacology of the gastric mucosa to improve drug penetration. New goals for therapy include altering motility, preventing adhesion and neutralising cytotoxin.

A novel intervention strategy was suggested by work from D O'Connor and A [Lee] (Australia) who showed in this first demonstration of bacterial interference in the gastric mucosa that G.hominis [eradicated] H.felis from the stomachs of infected mice. Treatments that do not clear H pylori in humans do not eradicate H.felis from mice.

An ad hoc committee was formed in Sydney to develop specific guidelines for in vitro susceptibility testing that can be accepted by all researchers in the field. If you are interested please contact: Dr T Ulf Westblom, Division of Infectious Diseases, St Louis University School of Medicine, 1402 S. Grand Boulevard, St Louis, MO 63104, USA or Dr Y Glupczynski, Brugmann University Hospital, 4 Place van Gehuchten, Brussels 1020 Belgium.

Gastric cancer link with H.pylori becomes stronger

Two papers recently published in the New England Journal of Medicine have dramatically strengthened the hypothesis that past infection with H.pylori considerably increases the risk of gastric carcinoma.

In the last issue of HP Worldwide, the first prospective study (by D Forman and colleagues) linking prior H.pylori infection to subsequent development of gastric cancer was reported. The latest studies in larger groups and in different populations have now confirmed and extended these initial findings.

In the Californian study (Parsonnet et al), 186 patients with gastric carcinoma were selected from nearly 129,000 individuals followed since the 1960's at a health maintenance organisation. The mean interval between serum collection and diagnosis was 14.2 years. Comparison of these patients with matched controls showed that 84% of the 109 patients with confirmed gastric adenocarcinoma had been infected previously compared with 61% of the controls (odds ratio, 3.6). H pylori infection was a particularly strong risk factor for women (odds ratio, 18) and blacks (9). A history of peptic ulcer disease was negatively associated with subsequent gastric cancer.

The authors conclude that individuals seropositive for H.pylori were approximately three times more likely to have gastric cancer in the ensuing 1 to 24 years of follow-up than matched controls.
In contrast to earlier studies from Parsonnet's group which suggested that intestinal type cancer but not the diffuse type was linked to H.pylori infection, no difference was seen between the odds ratios for the two types of cancer.

Is early infection important?

Only a very small proportion of infected persons ever develop gastric cancer and Parsonnet et al suggest that to increase the risk of cancer H pylori infection must begin in childhood. W Haenszel and coworkers had already shown in 1972 that the risk of gastric carcinoma was largely determined by environmental factors in the first few decades of life. H pylori infection in childhood is well known to be more common in high-risk populations.

H pylori is a plausible pathophysiologic cofactor for cancer, argue Parsonnet et al. Metabolic products of the organisms may directly transform the mucosa; rapid turnover of cells resulting from infection-induced injury may increase the risk of DNA damage, predisposing the mucosa to transformation by ingested or endogenous mutagens; endogenous byproducts of inflammation such as superoxide and hydroxyl ions may cause oxidative damage, mutation, and malignant transformation. Chronic inflammation in other organ systems has been linked with an increased cancer risk, e.g. ulcerative colitis and colorectal carcinoma.

High-risk population

The second paper focuses on a known gastric cancer high-risk population. A Nomura and coworkers examined serum samples collected between 1967 and 1970 from 5908 American men of Japanese ancestry in Hawaii. By 1989, 109 cases of pathologically confirmed gastric carcinoma had been diagnosed. 94% of these were positive for H pylori antibodies compared to 76% of the matched controls (odds ratio, 6.0). As in Forman's study, there was a progressive increase in the risk of gastric carcinoma as the level of H pylori antibody increased.

Both groups of investigators estimate that approximately 60% of gastric carcinomas in the populations studied could be preventable if H pylori infection were eliminated.

Association of H.pylori with gastric cancer beyond reasonable doubt

These findings attracted great interest at the recent conferences in Sydney and Amsterdam. "Gastric cancer now belongs in the spectrum of diseases that are associated with H pylori infection" said Dr David Forman (UK), speaking at the European Digestive Disease Week in Amsterdam.

While atrophic gastritis has long been associated with gastric cancer and epidemiological data have supported the idea of an association with H pylori, the publication of three follow-up studies in different populations has "changed the situation dramatically" said Forman. "It is now beyond reasonable doubt that there is some association between H pylori and gastric cancer."

Possible properties of H pylori that are relevant to carcinogenesis include sustained inflammation, cytokine expression, hypochlorhydria, reactive oxygen intermediates, decrease in gastric juice ascorbic acid and monocyte and macrophage activation. Forman drew attention to the work of Sobala and colleagues (reported in the last issue of HP Worldwide), showing a decrease in the amount of ascorbic acid in gastric juice with H pylori infection. "H pylori not only seems to be involved in generating carcinogens in the stomach" said Forman. "It also suppresses inhibition of the [illegible] by natural antioxidants such as ascorbic acid. The entire model of gastric cancer, particularly the link with dietary factors, is going to have to be rethought."

Dr Forman stressed that other [illegible] are involved in the development of gastric cancer. Strain differences may be responsible for the different [illegible] of gastric cancer. [illegible] addressed include possible [illegible] the [illegible] of infection, links with [illegible] and histology and primary prevention of infection.

Intervention trial

The next step in the chain of evidence is obviously an intervention trial [illegible] whether prevention [illegible] reduces incidence of gastric cancer.

One such trial currently under way is being carried out in Venezuela by the International Agency for Research on Cancer in association with the [illegible] Health Organisation. The Andean region of Latin America is one of the highest risk areas in the world for gastric cancer. Gastric cancer is the leading cause of death [illegible] cancer in Venezuela. An extensive screening program has been operating since early 1980s.

The aim of the study is to determine whether intervention [illegible] antioxidant treatment [illegible] H pylori, can block or reverse the progression of superficial gastritis [illegible] chronic atrophic gastritis [illegible] metaplasia. This series [illegible] to represent a continuum [illegible] normal to cancer.

Most dietary studies have shown [illegible] protective effect of a diet [illegible] vegetables and this provides the [illegible] for the use of the antioxidants [illegible] ne and vitamins E and C.

Subjects with a diagnosis [illegible] above lesions will be [illegible]

continued on page 6

in continuation of page 4

receive either anti-H pylori treatment or placebo. At the completion of this phase, the two groups will be further divided into those receiving antioxidant treatment or placebo. Because of the slow progression of precancerous lesions, results will not be available until 1995/6.

Is gastric cancer an infectious disease?

In an editorial accompanying the two latest papers in the New England Journal of Medicine, Dr P Correa (USA) poses the provocative question: Is gastric carcinoma an infectious disease? The cause of gastric cancer is almost certainly multifactorial, says Correa. Three factors appear to be dominant: H pylori infection, excessive salt intake and a diet low in fresh fruits and vegetables. Intervention studies currently under way should help to clarify the role played by H pylori.

Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. New England Journal of Medicine 1991; 325: 1127-31.

Nomura A, Stemmerman GN, Chyou P-H, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. New England Journal of Medicine 1991; 325: 1132-6.

Odds ratios for the association of gastric cancer with H pylori seropositivity

Study	Cases/controls	Odds ratio
Forman et al	29/116	2.9
Parsonnet et al	109/109	3.6
Nomura et al	109/109	6.0

Highlights on important developments in research on Helicobacter pylori

1. Graham DY et al.
The addition of triple therapy to ranitidine treatment accelerated healing in patients with duodenal ulcer (15)

2. Bertram TA et al
Patients from South America had a more severe gastritis than did those from North America. The neutrophil was the predominant inflammatory cell type in South American patients compared to the lymphocyte in North American patients. The authors conclude that the severity and cell type of H pylori associated gastritis are influenced by geographical factors that may be similar to those that modify infection rates for different geographical locations (313)

3. Karnes WE Jr et al
Serological data suggest that most patients with atrophic body gastritis, despite having a low incidence of current overt infection, have been infected with H pylori in the past (13)

4. Jones STM et al.
Presence of H pylori antibodies in rheumatoid arthritis patients correlated strongly with a previous history of peptic ulcer disease and with the severity of NSAID-related dyspeptic symptoms. The authors suggest that H pylori may have a definite role in the pathogenesis of symptomatic peptic ulcer disease associated with more chronic NSAID usage (21)

5. Morris AJ et al.
Arthur Morris describes how the H pylori infection he acquired after deliberately ingesting the organism was finally cleared by the use of triple therapy. Gastritis resolved and serological values returned to normal (14)

6. Blecker U et al.
A case is reported in a 15-year old girl of fatigue, pallor and syncopes, but no gastrointestinal symptoms, due to H pylori active haemorrhagic gastritis. On H pylori eradication, symptoms disappeared (38)

7. Oderda G et al
Recurrent abdominal pain, frequently associated with recurrent vomiting or diarrhoea, is the most common symptom of H pylori infection in children. One third of children have active gastritis which seems to be an early stage of the disease (2)

8. Sarosiek J et al.
The thickness of the mucus layer in biopsy material from the duodenum, antrum and corpus of H pylori infected subjects is significantly thinner than from control subjects (11)

9. Czinn SJ et al.
Oral immunisation of mice and ferrets with killed H pylori induced specific [illegible]

CALENDAR

1992	
25 - 27 March	British Society of Gastroenterology Meeting. Sheffield, UK
9 - 14 May	Digestive Disease Week. San Francisco, USA
19 - 23 May	34th Annual Meeting of the Hungarian Society of Gastroenterology Balaton, Hungary
5 - 7 July	Fifth Workshop on Gastroduodenal Pathology and Helicobacter pylori. Dublin, Ireland
25 - 30 September	1st United European Gastroenterology Week 14th International Congress of Gastroenterology 7th Congress of Digestive Endoscopy Athens, Greece
23 - 28 October	American College of Gastroenterology Miami Beach, Florida, USA
12 - 14 November	7th National Symposium of the Italian Society on Digestive Endoscopy Naples, Italy
27 - 28 November	Meeting on Upper Gastrointestinal Tract Diseases London, UK
29 November - 3 December	Asian Pacific Association for Gastroenterology Bangkok, Thailand
1993	
14 - 21 May	Digestive Disease Week Boston, USA
22 - 27 October	American College of Gastroenterology Washington DC, USA
1994	
21 - 27 May	Digestive Disease Week New Orleans, USA
2 - 7 October	10th World Congresses of Gastroenterology Los Angeles, USA

Bibliography

Recent Helicobacter pylori publications (up to December 1991) Completed from the Excerpta Medica database EMBASE and the Brocades Pharma literature system.

1 Debongnie JC, Donnay M. The role of endoscopy in the diagnosis of infections of the upper gastrointestinal tract. Acta Endosc 1991; 21/7 11-17

2 Oderda G, Vaira D. Helicobacter pylori infection in childhood. Acta Endosc 1991, 21/1 43-49.

3. Heringa CB. Report on the 3rd European symposium of the study group on Helicobacter pylori. Toledo (Spain), 9-10 November 1990. Acta Endosc 1991, 21/1 67-78.

4 Prewett EJ, Smith JTL, Nwokolo CU, et al. Eradication of Helicobacter pylori abolishes 24-hour hypergastrinaemia. A prospective study in healthy subjects. Aliment Pharmacol Ther 1991 5/3 283-290

5 Weil J, Bell GD, Powell K, et al. Omeprazole and Helicobacter pylori. Temporary suppression rather than true eradication. Aliment Pharmacol Ther 1991 5/3. 309-313.

6. Taha AS, Fraser WD, Kelly RW, et al. Inhibition of human gastric cyclic AMP production by Helicobacter pylori protein. Possible involvement of mucosal prostaglandin E2. Aliment Pharmacol Ther 1991, 5/4: 379-389.

7 Daw MA, Deegan P, Leen E, et al. Short report. The effect of omeprazole on Helicobacter pylori and associated gastritis. Aliment Pharmacol Ther 1991, 5/4, 435-439

8 Dooley CP. Helicobacter pylori: Review of research findings. Aliment Pharmacol Ther Suppl 1991, 5/1 129-143

9 Talley NJ. Non-ulcer dyspepsia: Myths and realities. Aliment Pharmacol Ther Suppl 1991, 5/1 145-162.

10 Nichols L, Sughayer M, DeGirolami PC, et al. Evaluation of diagnostic methods for Helicobacter pylori gastritis. Am J Clin Pathol 1991, 95/6, 769-773.

11 Sarosiek J, Marshall BJ, Peura DA, et al. Gastroduodenal mucus gel thickness in patients with Helicobacter pylori. A method for assessment of biopsy specimens. Am J Gastroenterol 1991 86/6 729-734

12 Lanza FL, Evans DG, Graham DY. Effect of Helicobacter pylori [illegible] gastroduodenal [illegible] acute administration [illegible] normal volunteers. Am J Gastroenterol [illegible] 86/6 735-737

13 Nensey YM, Schubert TT, [illegible] Helicobacter pylori-negative duodenal ulcer. Am J Med 1991 91 [illegible]

14 Morris AJ, Ali MR, [illegible] term follow-up [illegible] ingestion of Helicobacter pylori. Ann Intern Med [illegible] 114/9 662-663

15 Graham DY, Lew GM, Evans DG, et al. Effect of triple therapy (antibiotics plus bismuth) on duodenal ulcer healing. A randomized controlled trial. Ann Intern Med 1991 [illegible] 266-269

16 Jaskiewicz K, Louwrens HD. [illegible] atrophic gastritis in a population at risk for gastric carcinoma. Anticancer Res 1991 [illegible] 835-839

17 Counter FT, Ensminger PW, Preston DA, et al. Synthesis and antimicrobial [illegible] azithromycin (As-E 136 [illegible]) [illegible] macrolide antibiotic derived from erythromycin. Antimicrob Agents Chemother 1991 35/6 1116-1126

18 Mavromichalis I, Zaramboukas T, [illegible] Pl et al. Recurrent abdominal pain [illegible] gastrointestinal disorder. Arch Dis Child 1991 66/7 905

19 Chong CK. Underestimation of [illegible] pylori infection rate by biopsy urea breath test. Arch Intern Med 1991 151 [illegible] 462

20 Sturges HF, Hopkins RJ, Wasserman SS, et al. Helicobacter pylori in seventh day Adventists? Arch Intern Med 1991 151 [illegible] 1672-1674

21 Jones STM, Clague RB, Eldridge J, et al. Serological evidence of infection with Helicobacter pylori may predict gastrointestinal intolerance to non steroidal anti-inflammatory drug (NSAID) treatment in rheumatoid arthritis. Br J Rheumatol 1991 30 [illegible] 16-20

22. Forman D, Newell DG, Fullerton F, et al. Association between infection with Helicobacter pylori and risk of gastric cancer. Evidence from a prospective investigation. Br Med J 1991 302/6788 1302-1305

23 Baron JH, Holcombe C. Duodenal ulcer, gastric acid, and Helicobacter pylori. Br Med J 1991 302/6788 1333

24. Jankowski J. Helicobacter pylori infection and gastric cancer. Br Med J 1991 302/6791 1534

25 Heatley RV, Barrett J, [illegible] MF, et al. Helicobacter pylori infection and duodenal ulcer. Br Med J 1991, 302/6791 1534-1535

26. Bell GD, Powell K. Helicobacter pylori infection and duodenal ulcer. Br Med J 1991: 303/6798: 248.

27. Cougard J. Helicobacter pylori as a factor of gastric cancer. Cancer J 1991: 4/4 231.

28. Graham DY, Adam E, Reddy GT et al. Seroepidemiology of Helicobacter pylori infection in India. Comparison of developing and developed countries. Dig Dis Sci 1991: 36/8: 1084-1088.

29. Crabtree JE, Shallcross TM, Wyatt JI, et al. Mucosal humoral immune response to Helicobacter pylori in patients with duodenitis. Dig Dis Sci 1991. 36/9: 1266-1273.

30. Kozol R, Domanowski A, Jaszewski R, et al. Neutrophil chemotaxis in gastric mucosa. A signal-to-response comparison. Dig Dis Sci 1991 36/9 1277-1280.

31. Lee A, Fox JG, Otto G, et al. Transmission of Helicobacter spp. A challenge to the dogma of faecal-oral spread. Epidemiol Infect 1991: 107/1 99-109.

32. Kubonova K, Trupl J, Jancula L, et al. Presence of spiral bacteria (Gastrospirillum hominis) in the gastric mucosa. Eur J Clin Microbiol Infect Dis 1991· 10/5 459-460.

33. Hanninen ML. Sensitivity of Helicobacter pylori to different bile salts. Eur J Clin Microbiol Infect Dis 1991. 10/6. 515-518.

34. Glupczynski Y, Bourdeaux L, De Prez C et al. Prevalence of Helicobacter pylori in rural Kivu, eastern Zaire: A prospective endoscopic study. Eur J Gastroenterol Hepatol 1991: 3/6 449-455

35. Veldhuyzen Van Zanten SJO, Tytgat KMAJ, DeGara CJ, et al. A prospective comparison of symptoms and five diagnostic tests in patients with Helicobacter pylori positive and negative dyspepsia. Eur J Gastroenterol Hepatol 1991. 3/6 463-468.

36. Pieramico O, Ditschuneit H, Malfertheiner P. Oro-caecal transit time in patients with non-ulcer dyspepsia: Role of chronic gastritis and Helicobacter pylori infection. Eur J Gastroenterol Hepatol 1991: 3/8. 627-631.

37. Agnholt J, Fallingborg J, Moller-Petersen J, et al. The occurrence of Helicobacter pylori in the oesophagus. Eur J Gastroenterol Hepatol 1991. 3/9. 685-688.

38. Blecker U, Renders F, Lanciers S, et al. Syncopes leading to the diagnosis of a Helicobacter pylori positive chronic active haemorrhagic gastritis. Eur J Pediatr 1991: 150/8 560-561

39. Vaira D, Holton J, Barbara L. Helicobacter pylori and gastroduodenal disease. Gastroenterol Int 1991. 4/2 70-76

40. Eleftheriadis E, Kotzampassi K, Tzartinoglou E, et al. Colloidal bismuth subcitrate-induced changes on gastric mucosal hemodynamics in the rat. Gastric mucosal blood flow after CBS treatment. Gastroenterol Jpn 1991 26/3: 282 286.

41. Edge DP. Peptic ulcer in New Zealand. Gastroenterol Jpn 1991. 26/Suppl 3. 262-264.

42. Aceti A, Celestino D, Caferro M, et al. Basophil-bound and serum immunoglobulin E directed against Helicobacter pylori in patients with chronic gastritis. Gastroenterology 1991: 101/1. 131-137.

43. Karnes WE Jr, Samloff IM, Siurala M, et al. Positive serum antibody and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis. Gastroenterology 1991. 101/1. 167-174.

44. Griffith JL, Cummings OW, Hirschowitz BI. Development of sustained achlorhydria in a patient with the Zollinger-Ellison syndrome treated with omeprazole. Gastroenterology 1991. 101/1. 242-246.

45. George AA, Tsuchiyose M, Dooley CP. Sensitivity of the gastric mucosa to acid and duodenal contents in patients with nonulcer dyspepsia. Gastroenterology 1991. 101/1: 3-6.

46. Sugiyama T, Imai K, Yoshida H, et al. A novel enzyme immunoassay for serodiagnosis of Helicobacter pylori infection. Gastroenterology 1991: 101/1: 77-83.

47. Negrini R, Lisato L, Zanella I, et al. Helicobacter pylori infection induces antibodies cross-reacting with human gastric mucosa. Gastroenterology 1991: 101/2: 437-445.

48. Mooney C, Keenan J, Munster D, et al. Neutrophil activation by Helicobacter pylori. Gut 1991: 32/8: 853-857.

49. El Nujumi AM, Dorrian CA, Chittajallu RS, et al. Effect of inhibition of Helicobacter pylori urease activity by acetohydroxamic acid on serum gastrin in duodenal ulcer subjects. Gut 1991. 32/8: 866-870.

50. Neithercut WD, Milne A, Chittajulla RS, et al. Detection of Helicobacter pylori infection of the gastric mucosa by measurement of gastric aspirate ammonium and urea concentrations. Gut 1991: 32/9: 973-976.

51. Ferreres JC, Fernandez F, Vives AR, et al. Helicobacter pylori in Barrett's oesophagus. Histol Histopathol 1991: 6/3: 403-405.

52. Lapertosa G. Helicobacter pylori in Barrett's oesophagus. Histopathology 1991. 18/6: 568-570

53. Chan WY, Hui PK, Chan JKC et al. Epithelial damage by Helicobacter pylori in gastric ulcers. Histopathology 1991 19/1 47 53

54. Czinn SJ, Nedrud JG. Oral immunization against Helicobacter pylori. Infect Immun 1991. 59/7· 2359-2363.

55. Eaton KA, Brooks CL, Morgan DR, et al. Essential role of urease in pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets. Infect Immun 1991: 59/7 2470-2475.

56. Dunn BE, Sung CC, Taylor NS, et al. Purification and characterization of Helicobacter mustelae urease. Infect Immun 1991. 59/9· 3343-3345.

57. Vogt K, Rodloff AC, Hahn H. Combined activity of trospectomycin and colloidal bismuth subcitrate against Helicobacter pylori in vitro. Infection 1991. 19/3. 138-139

58. Gabay G, Pomeranz IS, Leichtmann G, et al. Helicobacter pylori infection - its prevalence and diagnosis in Israel. Isr J Med Sci 1991. 27/6 335-337

59. Drouet EB, Denoyel GA, Boude M, et al. Characterization of an immunoreactive species-specific 19-kilodalton outer membrane protein from Helicobacter pylori by using a monoclonal antibody. J Clin Microbiol 1991. 29/8 1620-1624

60. Talley NJ, Newell DG, Ormand JE, et al. Serodiagnosis of Helicobacter pylori: Comparison of enzyme-linked immunosorbent assays. J Clin Microbiol 1991. 29/8 1635-1639

61. Kiehlbauch JA, Plikaytis BD, Swaminathan B, et al. Restriction fragment length polymorphisms in the ribosomal genes for species identification and subtyping of aerotolerant Campylobacter species. J Clin Microbiol 1991 29/8 1670-1676.

62. Rivera E, Lopez-Vidal Y, Luqueno V, et al. Indirect immunofluorescence assay for detection of Helicobacter pylori in human gastric mucosal biopsies. J Clin Microbiol 1991. 29/8· 1748-1751.

63. Clayton C, Kleanthous K, Tabaqchali S. Detection and identification of Helicobacter pylori by the polymerase chain reaction. J Clin Pathol 1991. 44/6. 515-516.

64. Loffeld RJLF, Stobberingh E. Helicobacter pylori and ABO blood groups. J Clin Pathol 1991. 44/6. 516-517.

65. Queiroz DMM, Mendes EN, Rocha GA, et al. Helicobacter pylori and gastric histamine concentrations. J Clin Pathol 1991. 44/7· 612-613.

66. Goldie J, Veldhuyzen van Zanten SJO, Jalali S, et al. Inhibition of urease activity but not growth of Helicobacter pylori by acetohydroxamic acid. J Clin Pathol 1991: 44/8· 695-697

67. Ameglio F, Abbolito MR, Giannarelli D, et al. Detection of Helicobacter pylori carriers by discriminant analysis of urea and pH levels in gastric juices. J Clin Pathol 1991. 44/8: 697-698.

68. Crabtree JE, Mahony MJ, Taylor JD, et al. Immune responses to Helicobacter pylori in children with recurrent abdominal pain. J Clin Pathol 1991: 44/9: 768-771.

69. Logan RPH, Baron JH. Relationship between Helicobacter pylori hypergastrinaemia and duodenal ulcer. J Gastroenterol Hepatol 1991. 6/4: 417-418.

70. Hardikar W, Davidson PM, Cameron DJS, et al. Helicobacter pylori infection children. J Gastroenterol Hepatol 1991. 6/5: 450-454.

71. Debongnie JC, Pauwels S, Raat A, et al. Quantification of Helicobacter pylori infection in gastritis and ulcer disease using a simple and rapid carbon-14-urea breath test. J Nucl Med 1991. 32/6: 1192-1198.

72. De Giacomo C, Lisato L, Negrini R, et al. Serum immune response to Helicobacter pylori in children: Epidemiologic and clinical applications. J Pediatr 1991. 119/2: 205-210.

73. Assous M, Zone A, Watine J, et al. Frozen biopsy specimens and culture rates of Helicobacter pylori. Lancet 1991. 337/8754: 1412-1413.

74. Megraud F, Boyanova L, Lamouliatte H. Activity of lansoprazole against Helicobacter pylori. Lancet 1991: 337/8755: 1486.

75. Klein PD, Gilman R, Leon-Barua R, et al. Water source as risk factor for Helicobacter pylori infection in Peruvian children. Lancet 1991. 337/8756: 1503-1506.

76. Taylor DE, Salama SM, Chang N, et al. Isolation of novel microorganism from gastric biopsy specimen. Lancet 1991: 337/8756: 1542-1543.

77. Flocca R, Solcia E, Santoro B. Duodenal ulcer relapse after eradication of Helicobacter pylori. Lancet 1991. 337/8757· 1614.

78. Sobala GM, Crabtree JE, Pentith JA, et al. Screening dyspepsia by serology to Helicobacter pylori. Lancet 1991. 338/8759: 94-96.

79. Tompkins DS, Rotowa NA. Isolation of Helicobacter pylori from frozen gastric biopsy specimens. Lancet 1991. 338/8761 253.

80. Crabtree JE, Taylor JD, Wyatt JI, et al. Mucosal IgA recognition of Helicobacter pylori 120 kDa protein, peptic ulceration, and gastric pathology. Lancet 1991. 338/8763: 332-335.

81. Bateson MC, Chung CK, Lofeld RJLF, et al. Screening for Helicobacter pylori in dyspepsia. Lancet 1991. 338/8763: 393-394.

82. Hentschel E, Nemec H, Schutze K, et al. Duodenal ulcer recurrence and Helicobacter pylori. Lancet 1991: 338/8766: 569.

83. Hauke C, Grabner W, Grosse M, et al. Lymphatic follicles and intestinal metaplasia in the antral mucosa, a reaction by Helicobacter pylori infection. Leber Magen Darm 1991. 21/4· 156-160.

84. Mertz HR, Walsh JH. Peptic ulcer pathophysiology. Med Clin North Am 1991. 75/4 799-814.

85. Clearfield HR. Helicobacter pylori: Aggressor or innocent bystander? Med Clin North Am 1991. 75/4 815-829

86. Spiro HM. Some perspectives on peptic ulcer. Med Clin North Am 1991. 75/4 941-946

87. Rubin W. Medical treatment of peptic ulcer disease. Med Clin North Am 1991. 75/4. 981-998.

88. Janus G, Hammermeister I, Prignet JM, et al. Helicobacter pylori: a serological study of young soldiers with endoscopic, microbiologic and histologic study of ten cases with positive serology. Med Mal Infect 1991. 21/5 308 312.

89. Yokota K, Kurebuyashi Y, Takayama Y, et al. Colonization of Helicobacter pylori in the gastric mucosa of Mongolian gerbils. Microbiol Immunol 1991: 35/6 475-480

90. Johnson RC, Weil J, Bell GD, et al. Helicobacter pylori infection in uraemic patients. Nephron 1991. 58/3: 376.

91. Loffeld RJLF, Peltenburg HG, V/d Oever H, et al. Prevalence of Helicobacter pylori antibodies in patients on chronic intermittent haemodialysis. Nephron 1991. 59/2: 250-253.

92. Martinez E, Marcos A. Helicobacter pylori and peptic ulcer disease. New Engl J Med 1991. 325/10: 737-738.

93. Boleuardi C, Formentin PA. Peptic ulcer disease: Two cases in children. Pediatr Med Chir 1991: 13/2. 217-221.

94. Rota Zapata L, Molina Arias M, Rodriguez Balo A, et al. Helicobacter pylori. Pediatrika 1991: 11 May: 123-130.

95. Nakshabendi IM, Peebles SE, Lee FD, et al. Spiral shaped microorganisms in the human duodenal mucosa. Postgrad Med J 1991. 67/791· 846-847.

96. Axon A. H pylori and gastroduodenal disease. Practitioner 1991. 235/1507· 733-734+736.

97. Catrenich CE. Pathogenesis of infection by Helicobacter pylori: Introduction. Rev Infect Dis 1991: 13/Suppl 8: S655-S656.

98. Dunn BE, Altmann M, Campbell GP. Adherence of Helicobacter pylori to gastric carcinoma cells: Analysis by flow cytometry. Rev Infect Dis 1991: 13/Suppl 8 · S657-S664.

99. Karnes WE Jr, Ohning GV, Sytnik B, et al. Elevation of meal-stimulated gastrin release in subjects with Helicobacter pylori infection: Reversal by low intragastric pH. Rev Infect Dis 1991: 13/Suppl 8: S665-S670.

100. Fox JG, Otto G, Murphy JC, et al. Gastric colonization of the ferret with Helicobacter species: Natural and experimental infections. Rev Infect Dis 1991: 13/Suppl 8: S671-S680.

101. Krakowka S, Eaton KA, Rings DM, et al. Gastritis induced by Helicobacter pylori in gnotobiotic piglets. Rev Infect Dis 1991: 13/Suppl 8: S681-S685.

102. Leunk RD. Production of a cytotoxin by Helicobacter pylori. Rev Infect Dis 1991: 13/Suppl 8: S686-S689.

103. Murray PD. Pathophysiology and epidemiology of infection by Helicobacter pylori: Introduction. Rev Infect Dis 1991. 13/Suppl 8: S690.

104. Lambert JR. Pharmacology of bismuth-containing compounds. Rev Infect Dis 1991: 13/Suppl 8: S691-S695.

105. Elta GH. Helicobacter pylori in patients with nonulcer dyspepsia. Rev Infect Dis 1991: 13/Suppl 8: S696-S699.

106. Czinn SJ, Carr HS, Speck WT. Diagnosis of gastritis caused by Helicobacter pylori in children by means of an ELISA. Rev Infect Dis 1991: 13/Suppl 8: S700-S703.

107. Blaser MJ, Perez-Perez GI, Lindenbaum J, et al. Association of infection due to Helicobacter pylori with specific upper gastrointestinal pathology. Rev Infect Dis 1991: 13/Suppl 8: S704-S708.

108. Morgan DR, Costas M, Owen RJ, et al. Characterization of strains of Helicobacter pylori: One-dimensional SDS-PAGE as a molecular epidemiologic tool. Rev Infect Dis 1991: 13/Suppl 8: S709-S713.

109. Bertram TA, Krakowka S, Morgan DR. Gastritis associated with infection by Helicobacter pylori: Comparative pathology in humans and swine. Rev Infect Dis 1991. 13/Suppl 8: S714-S722.

110. De Korwin JD, Bene MC, Duprez A, et al. Helicobacter pylori gastritis, a new pathological entity. Significant reduction of inflammatory infiltration after antibiotic therapy. Rev Med Interne 1991. 12/Suppl: 566.

111. Dick-Hegedus E, Lee A. Use of mouse model to examine anti-Helicobacter pylori agents. Scand J Gastroenterol 1991. 26/9: 909-915.

112. Malaty HM, Graham DY, Klein PD, et al. Transmission of Helicobacter pylori infection. Studies in families of healthy individuals. Scand J Gastroenterol 1991. 26/9: 927-932.

113. Bertram TA, Murray PD, Morgan DR, et al. Gastritis associated with infection by Helicobacter pylori in humans: geographical differences. Scand J Gastroenterol Suppl 1991 26/181. 1-8.

114. Correa P, Ruiz B, Hunter F. Clinical trials as etiologic research tools in Helicobacter-associated gastritis. Scand J Gastroenterol Suppl 1991 26/181 15-19

115. Costas, M, Owen RJ, Bickley J, et al. Molecular techniques for studying the epidemiology of infection by Helicobacter pylori. Scand J Gastroenterol Suppl 1991. 26/181 20-32.

116. Czinn SJ, Bertram TA, Murray PD et al. Relationship between gastric inflammatory response and symptoms in patients infected with Helicobacter pylori. Scand J Gastroenterol Suppl 1991 26/181 33 37

117. Eaton KA, Radin MJ, Kramer L, et al. Gastric spiral bacilli in captive cheetahs. Scand J Gastroenterol Suppl 1991. 26/181 38-42

118. Owen RJ, Bickley J, Costas M, et al. Genomic variation in Helicobacter pylori: Application to identification of strains. Scand J Gastroenterol Suppl 1991: 26/181 43-50.

119. Recavarren-Arce S, Leon-Barua R, Cok J, et al. Helicobacter pylori and progressive gastric pathology that predisposes to gastric cancer. Scand J Gastroenterol Suppl 1991: 26/181 51-57.

120. Catrenich CE, Makin KM. Characterization of the morphologic conversion of Helicobacter pylori from bacillary to coccoid forms. Scand J Gastroenterol Suppl 1991: 26/181· 58-64.

121. Goggin PM, Northfield TC, Spychal RT. Factors affecting gastric mucosal hydrophobicity in man. Scand J Gastroenterol Suppl 1991· 26/181: 65-73.

122. Crave TR, Cave DR. Helicobacter pylori stimulates pepsin secretion from isolated rabbit gastric glands. Scand J Gastroenterol Suppl 1991: 26/181: 9-14

123. Bertram TA. Second annual symposium on Helicobacter pylori: Foreword. Scand J Gastroenterol Suppl 1991 26/181 V

124. Tytgat GNJ, Noach LA, Rauws EAJ. Is gastroduodenitis a cause of chronic dyspepsia? Scand J Gastroenterol Suppl 1991 26/182: 33-39.

125. Lee SP. The mode of action of colloidal bismuth subcitrate. Scand J Gastroenterol Suppl 1991: 26/185: 1-6.

126. Lam S-K. Treatment of duodenal ulcer with sucralfate. Scand J gastroenterol Suppl 1991. 26/185: 22-28.

127. Benet LZ. Safety and pharmacokinetics: Colloidal bismuth subcitrate. Scand J Gastroenterol Suppl 1991. 26/185: 29-35.

128. Holcombe C, Kaluba J, Lucas SB. Non-ulcer dyspepsia in Nigeria: A case-control study. Trans R Soc Trop Med Hyg 1991. 85/4 553 555.

129. Holcombe C. Aetiology and management of dyspepsia in Africa. Trop Doct 1991. 21/3· 107-112.

130. Al-Quorain A, Satti MB, Al-Hamdan A, et al. Pattern of upper gastrointestinal disease in the eastern province of Saudi Arabia. Endoscopic evaluation of 2.982 patients. Trop Geogr Med 1991. 43/1-2. 203-208

131. Ofusanya O. Immunological response to Helicobacter pylori among Nigerians. Trop Geogr Med 1991. 43/1-2: 28-32

132. Kang JK, Kim E, Kim KH, et al. Association of Helicobacter pylori with gastritis and peptic ulcer diseases. Yonsei Med J 1991. 32/2· 157-168.

Changing views on duodenal ulcer treatment

"All duodenal ulcer patients should now be offered H.pylori eradication therapy", said Dr Tom Borody (Australia) at a recent symposium in Amsterdam on 'The disease consequences of H.pylori infection in man'. "I feel that in future H_2-blockers will form second line therapy."

While not everyone agreed completely with Dr Borody's recommendations, the majority of the audience attending the symposium came away with changed views on how to treat duodenal ulcer.

"Should we now be concentrating on pH or Hp, on acid suppression or H.pylori eradication", asked Dr Borody. "There is very little doubt that H.pylori eradication virtually abolishes duodenal ulcer recurrence and changes the natural history of the disease", he said, referring to follow-up studies over six years. H.pylori has been shown to be the major causal factor in over 94% of duodenal ulcer patients in his clinic.

"Suppression of acid by H_2-blockers is unphysiological", pointed out Borody. "Relapse rates of 75-89% after stopping therapy may necessitate costly life-long therapy. On the other hand, eradication of H.pylori aims to restore normal physiological conditions. Therapy is cheap, need only last for two weeks and results in accelerated ulcer healing and prevention of ulcer relapse."

Optimising triple therapy

How can triple therapy be optimised? "What is important is the drug concentration at the mucosal surface", explained Borody. "The side effects are probably due to systemic absorption of antibiotics. Individual doses can be lowered by increasing the frequency while at the same time increasing the local concentration by the use of H_2-blockers or proton pump blockers." Dr Borody reported that increasing the frequency of triple therapy dosing to five times a day, halving the tetracycline concentration and reducing metronidazole from 250 to 200 mg, and adding an H_2-blocker at night, resulted in a significant reduction in side effects without any effect on the rate of eradication, around 95%.

What is the future role of H_2-blockers in duodenal ulcer disease? "These drugs will still be used in temporary treatment and in pain reduction during triple therapy", said Dr Borody. "They will continue to be used as maintenance treatment where treatment has failed to eradicate H.pylori and may be used to optimise triple therapy by increasing the local concentration of antibiotics."

How to treat patients in whom eradication has previously failed? Dr Borody uses a custom-built therapy based on drug sensitivity and achieves 70% eradication in this patient group. "Metronidazole resistance is not a significant factor", he claimed. "Eradication rates of 87% are still achieved in the population carrying resistant strains."

"It is going to become unethical not to offer antibacterial therapy to patients with chronic duodenal ulcer", said Dr Borody.

Dr Tony Axon (UK), co-chairman of the symposium, outlined other major unanswered questions. If H.pylori is so widespread why doesn't everyone have duodenal ulcer? How does the organism cause ulcer? What evidence is there that H.pylori may be responsible for gastric cancer?

Professor Martin Blaser (USA) spoke on pathogenic mechanisms involved in H.pylori infection (see page 1) and the role of H.pylori in gastric carcinogenesis was reviewed by Dr David Forman (UK)(see page 4).

Different disease outcomes

Dr Mike Dixon (UK) focused on those morphological features of gastritis that give some insight into the natural history of the disease and disease associations.

Acute neutrophilic gastritis is rarely seen at biopsy. Three months after infection the gastritis has become chronic with infiltrates of chronic inflammatory cells. In only very few individuals does the gastritis resolve. 80% of those with gastritis go on to develop chronic pangastritis, which may develop into atrophic gastritis, both the prevalence and severity of which increase with age. Intestinal metaplasia also increases with age. In wide-spread atrophy and intestinal metaplasia, H.pylori is no longer present.

Chronic pangastritis with continuing epithelial degeneration is associated with gastric ulcer and atrophic gastritis is associated with gastric cancer. "However the vast majority of subjects have no symptoms", pointed out Dr Dixon.

The remaining 20% of individuals infected with H.pylori develop antral gastritis, which is associated with gastric metaplasia, active chronic duodenitis and duodenal ulcer. The occurrence of gastric metaplasia is not dependent on H.pylori infection but is more extensive in infected individuals. "Gastric metaplasia appears to develop as a response to an increased acid load", explained Dr Dixon.

What determines which of these two patterns of inflammation occurs? Dr Dixon speculated that in individuals with a high intrinsic acid output, the acid in the corpus may protect against colonisation. Such individuals will develop an antral predominant gastritis and may develop gastric metaplasia in the duodenum because of the high acid load reaching the first part of the duodenum. Antral gastritis and gastric metaplasia have long been known as the two major risk factors in duodenal ulcer.

"The central feature leading to diminished mucosal resistance is chronic inflammation. The only way to reduce inflammation is to remove its cause, to eradicate H.pylori", concluded Dr Dixon.

The most common chronic infection in man

H.pylori eradication also received attention in other sessions.

"There is no doubt that H.pylori has revolutionised our thinking about the etiology and treatment of duodenal ulcer", said JJ Misiewicz (UK), reviewing recent advances in gastroenterology. "The prolonged hypergastrinemia associated with H.pylori infection, the 'most common chronic infection in man', may lead to increased parietal cell mass, hyperacidity and resultant gastric metaplasia in the duodenum and subsequent colonisation of this gastric metaplasia by H.pylori from the stomach", suggested Dr Misiewicz. "Triple therapy is the only way to eradicate H.pylori", he said, "and the trend is towards shorter and shorter treatment regimens." A one week treatment regimen was reported by RPH Logan (UK). Duodenal ulcers healed in 23/33 patients after only one week of triple therapy and 7 of the remaining 11 went on to heal after this week. The overall eradication rate was 58%.

"Eradication of H.pylori should be considered before surgery", recommended RH Hunt (Canada), speaking on the long-term therapeutic options for peptic ulcer disease.

Is H.pylori involved in the development of refractoriness to H_2-antagonist treatment? G Bianchi Porro (Italy) reported that 96% of duodenal ulcer patients resistant to 4 weeks full dose H_2-blocker treatment healed after triple therapy compared to 31% after sucralfate. 8/9 patients who did not heal with sucralfate subsequently healed with triple therapy. One month after healing, eradication of H.pylori was 83% in the treated group but nil in the sucralfate group. On the basis of these results Bianchi Porro suggested that first line therapy with triple therapy instead of H_2-blockers should be considered.

Less relapse in gastric ulcer

Most eradication studies have focused on duodenal ulcer. K Seppala (Finland) presented early results showing that H.pylori eradication improves the rate of healing in gastric ulcer. Healing was seen in 92% of cases where H.pylori was eradicated compared to 74% who remained H.pylori-positive. Relapse data confirm what has been shown in duodenal ulcer. None of the 15 patients who remained H.pylori-negative had relapsed after 33 weeks compared to 14/38 who were H.pylori-positive.

Seeing H.pylori in vivo

Direct visualisation of H.pylori on the human gastric mucosa was reported by T Kato and colleagues (Japan), using an in vivo urease test. The patchy distribution of H.pylori on the mucosa has been a problem in diagnosis by biopsy. After premedication with famotidine or omeprazole to adjust the gastric pH, the gastric mucosa was sprayed with a mixture of phenol red and urea by a tube inserted through the endoscope. H.pylori-positive areas changed colour. Biopsies confirmed the presence or absence of H.pylori, with 2/37 false positives and no false negatives.

Seeing H.pylori through the microscope may not be quite so easy. AH Christensen (Denmark) warned of the limitations of histological diagnoses of H.pylori infection by comparing the reproducibility of diagnoses by different pathologists.

1991

Chapter 17

GONORRHEA VACCINES

John W. Boslego and *Carolyn D. Deal*

ETIOLOGIC AGENT AND PATHOGENESIS

Description of the Agent

Neisseria gonorrhoeae is the etiologic agent of gonorrhea. The bacterium belongs to the family Neisseriaceae, which includes both pathogenic *(N. meningitidis)* and nonpathogenic *(N. sicca, N. subflava) Neisseria* (1). The clinical manifestations of gonorrhea, such as genital exudates, have been described for centuries, but it was not until 1879 that Albert Neisser first described the organism in urethral pus (2). *Neisseria gonorrhoeae* was first isolated in vitro in 1882.

Neisseria gonorrhoeae is a gram-negative diplococcus that grows on artificial medium at 37°C in a 5% CO_2 environment. The organism is oxidase-positive and ferments glucose, but not maltose, sucrose, or lactose.

Satisfactory treatment of clinical gonococcal infections was first achieved in the 1930s with sulfonamides (3). However, the organism quickly developed resistance to the drug, which has been a recurring theme. Penicillin was introduced for *N. gonorrhoeae* therapy in the 1940s and was highly successful (4). In the past 40 years, however, the organism has continued to evolve and manifest a variety of drug resistances (5). Penicillin can no longer be used in many regions of the world, including parts of the United States (5). Close surveillance of antimicrobial susceptibility patterns is necessary since the organism continues to develop resistance to the drugs used for its treatment.

Clinical Manifestations

Neisseria gonorrhoeae usually causes a local mucosal infection, but the spectrum of disease ranges from asymptomatic carriage to disseminated infection. The primary manifestations are urethritis in the male and cervicitis in the female. *Neisseria gonorrhoeae* infects other mucosal surfaces as well, resulting in conjunctivitis (neonatal and adult), pharyngitis, and proctitis.

Local extension of infection occurs in both sexes and is responsible for the major morbidity associated with gonorrhea. In men, the infection may extend to the epididymis, testes, or prostate. These unusual complications can result in sterility.

In the female, local extension of infection is more common, and more serious. Often acting in concert with other organisms, *N. gonorrhoeae* causes endometritis, salpingitis, peritonitis (pelvic inflammatory disease, PID), and perihepatitis. These infections can result in tubal scarring with infertility and subsequent ectopic pregnancy, tuboovarian abscess, and chronic pelvic pain. Estimates are that up to 45% of the women who contract a genital gonococcal infection will develop PID (6). Infertility rates after a single episodes of PID approach 15%, and after three episodes, 75% (6).

Disseminated gonococcal infection is manifested by papular or petechial skin lesions (usually on extremities), arthralgias, tenosynovitis, and oligoarthritis. Rarely, myocarditis, hepatitis, endocarditis, and meningitis occur. Strains causing disseminated infection are usually serum-resistant and of a particular auxotype (Arg^-,Ura^-,Hyx^-) (7). Patients who are genetically deficient in one of the terminal complement components are predisposed to repeated episodes of disseminated gonorrhea (8).

Epidemiology/Disease Burden

Gonorrhea is the most commonly reported infectious disease in the United States (6). Despite the relative ease of diagnosis and treat-

ment, and the extensive public health network for case identification and contact tracing, the epidemic continues.

In the United States, there are approximately 1 million cases reported each year (6). The highest attack rates are in young adults, ages 15 to 24 (6). It is estimated that the reported cases represent only about half of the actual cases. The number of cases peaked in the United States in 1975 and has plateaued and slowly declined since then (7).

Reliable estimates of disease rates worldwide are not available. Because many of the less-developed countries lack the resources for early diagnosis, treatment, and contact tracing, gonorrhea presents enoromous health problems in these regions. Epidemic infertility occurs in sub-Saharan Africa and is attributed to gonorrhea (6).

In the United States, the major morbidity and financial costs are related to PID. There are an estimated 400,000 cases of gonococcal PID each year. Considering the total number of cases of gonorrhea, the cost for evaluation and treatment (particularly of PID, infertility, and ectopic pregnancy) approaches $1 billion/year in the United States alone (6).

Pathogenesis

Neisseria gonorrhoeae is a uniquely human pathogen. The organism survives poorly outside the host unless artificially cultured. The disease is spread from person to person, usually by sexual contact with infected secretion. Once the bacteria are deposited on a mucosal surface, a series of events occurs that results in invasion of mucosal columnar cells and a host inflammatory response.

The stages of pathogenesis have been most closely studied in organ cultures of fallopian tubes and entail distant attachment of the organism to the host cell, close attachment and multiplication, ingestion by the epithelial cell, transportation through the cell in phagosomes, possible egestion onto the basement membrane, and, in rare instances, bloodstream invasion (9–11). The epithelial lining is markedly affected by gonococci, demonstrating loss of ciliary motility and extrusion of ciliated cells. The classic exudate consists mainly of host inflammatory cells, denuded epithelial cells, and gonococci.

The contribution of individual components of *N. gonorrhoeae* to each phase of pathogenesis is the subject of considerable study and will be discussed in more detail below. Recent developments in molecular biology have added substantially to our understanding of these events, but much remains to be learned.

HISTORY OF VACCINATION AGAINST GONORRHEA

Several gonococcal vaccines have been evaluated for efficacy in humans. The first effort was conducted by Greenberg et al. in Canada in the early 1970s (12). Greenberg et al. utilized three seed strains of type I (piliated) gonococci to prepare a killed, autolyzed vaccine. The vaccine was well tolerated and in the majority of volunteers stimulated an antibody response (bentonite flocculation, tissue culture neutralization) that was generally short-lived. Volunteers received three doses of vaccine, 1 week apart. In the study, conducted in a high-risk population, 62 volunteers were entered into a randomized, placebo-controlled trial. During a 12-month observation period, 10 of 33 vaccine recipients and 7 of 24 control recipients acquired gonorrhea, indicating no protective effect.

Brinton et al. later prepared purified pilus vaccines and tested them in human volunteers in a series of experimental challenge studies (13). The single-pilus vaccine was highly successful in preventing disease when the challenge strain was identical to the vaccine seed strain. The protection could be overcome by higher challenge inocula. Importantly, when a heterologous challenge strain was used, there was no apparent protection (C. Brinton, oral communication, 1982).

A field trail utilizing a purified single-pilus vaccine was conducted in high-risk U.S. military personnel in the Republic of Korea in 1983 (14). In this randomized double-blind, placebo-controlled trial, 3250 volunteers participated. Two doses of vaccine or placebo were administered 2 weeks apart. The observation period was 8 weeks. In male volunteers, 108 vaccine and 101 placebo recipients acquired gonorrhea 2 weeks or more after initial immunization. There was no vaccine protection despite the development of high levels of serum cross-reactive pilus antibody levels.

Lastly, a protein I vaccine was prepared and tested by E. W. Hook III in a human challenge model. The vaccine was well-tolerated and elicited a serum antibody response. This vaccine also afforded no protection against experimental gonorrhea (E. W. Hook III, oral communication, 1986).

VACCINE POTENTIAL OF IMPORTANT GONOCOCCAL ANTIGENS

Pilin or Peptides

Pili, filamentous projections from the cell, are one of the major surface antigens of the gonococcus. Each pilus is formed by the specific association of thousands of pilin protein subunits. This antigen displays both phase and antigenic variation. The organism can "switch" between a piliated and a nonpiliated state. Moreover, the subunits can display tremendous antigenic variation. The amino-terminal portion of the pilin sequence is highly conserved. Antigenic variation occurs predominantly in the carboxy-terminal region by insertions and deletions of two to four amino acid residues as well as single amino acid changes (15). The conserved amino-terminal sequence of the gonococcal pilin is homologous to that of other bacterial pilins including those from *N. meningitides* (16), *Pseudomonas aeruginosa* (17), *Moraxella nonliquefaciens* (18), and *Bacteroides nodosus* (19). All these sequences begin with the unusual N-methylphenylalanine residue.

Several studies have implicated pili as a virulence factor and as a potential vaccine candidate. Initial studies by Kellogg et al. reported that virulence in a human challenge model was correlated with certain colony morphologies (T1, T2) that were later discovered to represent piliated phenotypes (20, 21). Electron microscopic studies have implicated pili as mediating attachment of the gonococcus to the microvilli of nonciliate columnar epithelial cells (22). The pilus may somehow enable the organism to overcome the normal repulsive electrostatic barrier to attachment between the gonococcus and the host cell (23). Alternatively, a specific receptor–ligand interaction is possible, though no specific eukaryotic receptor for the pilus has yet been identified. Certainly, the presence of pili enhances attachment to a variety of eukaryotic cells. In addition to adherence, piliated gonococci have also been reported to be more resistant to phagocytosis than nonpiliated organisms (24).

Brinton et al. demonstrated that immunization of human volunteers with purified pilin resulted in the generation of antibody that was protective against challenge with the homologous strain (13, 25). In another human infection study, the expression of the pili was again correlated with virulence of the gonococcus, with piliated organisms causing disease and nonpiliated ones being avirulent (26). More importantly, this study demonstrated the in vivo antigenic variability of pilin. Disease isolates expressed numerous and different pilin types compared with pilin of the input strain. It is this capacity for antigenic variation in vivo that may allow the organism to circumvent a pilus type–specific antibody response.

The molecular mechanisms for antigenic variation have been studied by several laboratories (27–29). The variation is mediated by silent copies of pilin genes of differing antigenic types and locations on the gonococcal chromosome. Gene conversion of these copies into the expression site gives rise to expressed pilin of different antigenic types (30). One method of phase variation is deletion of the pilin gene at the expression site, which results in a nonpiliated phenotype, which can sometimes revert back to a piliated form (29). Recent reports indicate that another mechanism may also be involved, and that is DNA transformation of pilin genes between organisms (31). Thus, the gonococcus displays several sophisticated mechanisms for variation of this major surface antigen.

Selective regions of the pilin sequence, presented to the immune system as synthetic peptides, may make effective immunogens (32). Hopefully, a polypeptide representing only a sequence-conserved region may allow the resultant antibodies to effectively block all gonococci, but that is still speculative at this point. Studies to determine the x-ray crystallographic structure of the pilin molecule should facilitate the identification of exposed regions, which could be effective in this regard (33).

Pili, then, appear to be a major virulence factor for the gonococcus. The concept of a vaccine composed of pilin, or some portion thereof, is of considerable interest, but optimism for its success is dampened by the degree of pilin antigenic diversity and the fact that the human immune response appears directed primarily against the variable portion (14).

Nonpilin Adhesins

In addition to pilin, Muir et al. have reported the presence of other proteins associated with the pilus fiber (34). These proteins copurify with pilin and may possibly be incorporated into the pilus supramolecular structure. It is suggested that these proteins may be analogous to those seen in uropathogenic *Escherichia coli*, in which proteins incorpo-

rated at the tip of the pilus mediate adhesion to carbohydrate receptors present in the urinary tract (35).

Other studies indicate the possibility of pilin-independent adhesins that may be present on gonococci and mediate binding to eukaryotic cells. Both piliated and nonpiliated gonococci bind to carbohydrate-containing glycolipids (gangliotetraosylceramide and gangliotriaosylceramide) that have been isolated from eukaryotic cells (36). If this function is biologically significant and common among gonococcal strains, then an adhesin protein, or a peptide corresponding to the binding domain, would make a feasible vaccine candidate. However, the role of these proteins in the pathogenic process is unclear at present, and little is known about their potential immunogenicity.

Outer Membrane Proteins

There are three predominant outer membrane proteins (proteins I, II, III) in the gonococcal membrane. The role of these proteins in the pathogenesis of disease is still speculative. Proteins I and II are antigenically variable, while protein III appears to be identical in all strains.

Protein I

Protein I accounts for the majority of protein in the outer membrane and is designated the major outer membrane protein (37). It is found in all gonococci and varies in molecular mass (32–37 kDa) among strains (9). Protein I is believed to function as the porin protein by forming hydrophilic channels through the outer membrane (38). At least a portion of the protein is surface-exposed (39).

The antigenic variability of protein I provides a useful mechanism to classify gonococcal strains. A single strain expresses only one protein I, which remains antigenically stable (9). There are two major subclasses of protein I: protein IA and protein IB. The amino acid sequence of representative strains of each subclass is now known (40, 41). Each subclass represents a family of structurally different, but similar, protein I's. Protein IAs are generally of lower molecular weight. While the amount of protein I that is surface-exposed tends to differ among strains, protein IAs have a smaller surface-exposed portion than protein IBs (42). The surface-exposed determinants form the basis of the current serologic classification schemes. A commonly used system employs six protein IA and six protein IB monoclonal antibodies (43). On the basis of its reaction pattern to this panel of monoclonal antibodies in a coagglutination assay, a strain can be classified into a serovar. To date, 24 protein IA and 32 protein IB serovars are recognized worldwide. In any given region, however, the overwhelming majority of strains are represented by far fewer serovars.

There are functional correlations between protein I subclass/serovar and characteristics of the organism and/or expression of disease. Protein IAs are associated with disseminated gonococcal infection and resistance to killing by normal human serum (44). Protein IBs are more closely associated with antibiotic resistances (45) and with local mucosal disease (46). However, these associations are far from absolute, and there is considerable overlap. Nevertheless, this typing scheme has been of considerable value in outbreak investigations and in epidemiologic studies of disease transmission (47–49).

Although the role of protein I in the pathogenicity of human infection is not established, there are several lines of evidence that encourage its further investigation as a vaccine candidate.

Protein I is essential for organism survival. It is surface-exposed and invariant in a given strain. There is considerable structural and antigenic similarity among strains in each subclass. It appears to interact at the eukaryotic cell membrane and may trigger endocytosis of the organism by the host mucosal cell (9).

Protein I is immunogenic in humans during the course of a natural infection. Patients develop protein I antibodies in their local secretions and serum as a consequence of infection (50–53). These antibodies exhibit both opsonic and bactericidal properties (54, 55). Moreover, many of the protein I monoclonal antibodies activate complement and lyse the organism (42, 55).

Clinical studies also support the concept that protein I antibodies may protect against infection. Buchanan et al. demonstrated that recurrent episodes of acute gonococcal salpingitis were not caused by gonococci of the same protein I type (56). More recently, Plummer et al. showed that female genital infection with a given serovar appeared to provide protection against a subsequent infection with the same serovar (57).

To date, one protein I vaccine was unsuccessful in a male gonococcal urethritis infection trial, as described previously. While dis-

appointing, this tr
investigation of alt
Manufacturing a
ture of protein I se
a quicker avenue
lies in the identifi
tope(s) that is surf
of functional antil

The current
protein I sequen
tion on surface-e
ods for gene cl
greatly enhance o
portant antigen (

Protein II

Protein II is a
able outer meml
actually describe
(protein IIs) that
strain and interst
tein is at least
When expressed
percentage of th

Protein II exp
in part, with the
velop opaque co
culture media a
microscope (61)

The antigeni
been the subject
gle strain may
than one antige
and switch from
tein II–negative
tein II type to
(59, 62).

Protein II ap
herence proper
II expression is
cocci–gonococ
creased adhes
epithelial cells
trophils (64). I
tein IIs medi
types.

The role o
in unknown.
opaque varia
ered from n
whereas tran
recovered fr
nated infecti
nogenic in n
females deve
terial proper
tigenic varia
vivo. In an

appointing, this trial should not impede the investigation of alternative protein I vaccines. Manufacturing a vaccine composed of a mixture of protein I serovars is one approach, but a quicker avenue for vaccine development lies in the identification of a conserved epitope(s) that is surface-exposed and the target of functional antibody activity.

The current or imminent availability of protein I sequence data, structural information on surface-exposed epitopes, and methods for gene cloning and expression will greatly enhance our understanding of this important antigen (40, 41, 58).

Protein II

Protein II is a 24- to 30-kDa heat-modifiable outer membrane protein (9). Protein II actually describes a family of related proteins (protein IIs) that manifest tremendous intrastrain and interstrain variations (59). The protein is at least partially surface-exposed. When expressed, protein II constitutes a high percentage of the outer membrane (60).

Protein II expression is associated, at least in part, with the ability of the organism to develop opaque colony types when grown on culture media and viewed under a dissecting microscope (61).

The antigenic variation of protein II has been the subject of considerable study. A single strain may simultaneously express more than one antigenically different protein IIs and switch from a protein II–positive to protein II–negative phenotype, or from one protein II type to another at a high frequency (59, 62).

Protein II appears to bestow increased adherence properties to gonococci (63). Protein II expression is associated with greater gonococci–gonococci adhesion, as well as increased adhesion of gonococci to human epithelial cells, conjunctival cells, and neutrophils (64). It is possible that different protein IIs mediate adhesion to different cell types.

The role of protein II in human infection in unknown. Clinical studies have shown that opaque variants are more commonly recovered from mucosal gonococcal infections, whereas transparent variants are more often recovered from asymptomatic or disseminated infections (65). Protein IIs are immunogenic in natural infection. Both males and females develop antibodies, but their antibacterial properties are not known (50, 66). Antigenic variation of protein IIs also occurs in vivo. In an experimental infection study, a broad array of protein II variants appeared during the course of infection after a predominately protein II–negative phenotype was instilled intraurethrally (67).

Protein II is currently not considered an attactive vaccine candidate. Although it is surface-exposed and quantitatively significant in protein II–positive organisms, the bacteria can survive and grow in its absence. Even though a pathogenic relationship is suggested by its adherence properties, too little is currently known to establish a defined role in human infection. Lastly, the phenomenal array of antigenically distinct protein IIs would make it a very difficult antigen to incorporate into a vaccine.

Protein III

Protein III is a 30- to 31-kDa outer membrane protein (9). It is surface-exposed and present in all strains of gonococci (68). Moreover, an analogous protein, designated class 4, also exists in *N. meningitidis* (69).

Protein III is closely associated with protein I in the bacterial membrane, but its function in bacterial physiology or in the pathogenesis of disease is not known (69). There is no evidence to support its role as a porin protein or as a cofactor for protein I in this capacity.

In stark contrast to other gonococcal surface antigens, there is no evidence for structural or antigenic variation in protein III (70). All avaliable studies suggest it is invariant among gonococcal strains. Moreover, protein III shares remarkable sequence and structural similarity to the Omp A proteins of *Enterobacteriaceae*, particularly *E. coli* (71). This homology is especially marked in the carboxy portion of the molecule (69).

Despite its surface location, protein III is poorly immunogenic in humans during natural infection (50, 66). Patients demonstrate either no response or low levels of antibody.

The most fascinating aspect of protein III is its apparent capacity to induce and/or bind to antibodies that block the bactericidal activity of antibodies to other surface antigens (protein I, lipopolysaccharide) (72, 73). There is experimental evidence that protein III antibodies (IgG) fix complement, but the resultant membrane attack complex is either defective or incapable of fully inserting into the cell membrane to cause bacterioloysis (74, 75).

Due to its antigenic similarity to Omp A proteins, patients may develop protein III antibodies as a result of colonization/infection

by *Enterobacteriaceae* These cross-reactive protein III antibodies may then shield the gonococcus from the bactericidal activity of other antibodies. Protein III might then exist on the gonococcus as a mechanism for its own protection. If this scenario proves valid, protein III might be deleterious if incorporated into a vaccine preparation.

Considerable work is in progess to resolve these important issues. The recent construction of a protein III–deficient gonococcal strain will significantly aid in this evaluation (69).

Lipopolysaccharide

Lipopolysaccharide (LPS) is a major constituent of gram-negative outer membranes and is known to serve several important biological and pathogenic functions. In addition, various LPS epitopes, distinguishable by monoclonal antibodies, are expressed on different gonococcal strains or on the same strain at different times, resulting in LPS antigenic variation.

Phenol-extracted LPS has been shown to mediate most of the toxic damage that occurs during infection of human fallopian tubes (76). The LPS is a target for bactericidal antibodies and regulates complement activation on the bacterial cell surface (77, 78). The presence or absence of certain LPS epitopes may be involved in the determination of serum-sensitive or serum-resistant phenotypes (79).

The structure of gonococcal LPS is similar to that of enteric bacteria in that both have a lipid A fatty acid chain embedded into the cell wall, and a core oligosaccharide linked to three 3-deoxymanno-2-ketooctulosonic acid (KDO) moieties. The gonococcus differs from enteric bacteria, however, in that it lacks an *O* side chain of strain-specific polysaccharide residues (80). Silver staining of periodate-oxidized LPS and rapid isolation methods have enabled the determination of LPS molecular masses ranging from 3.2 to 7 kDa among strains (81). The development of LPS monoclonal antibodies has allowed the immunochemical characterization of specific LPS components (79). Recently the structural determination for the oligosaccharide portion of the gonococcal LPS has been proposed (82). Studies by Mandell et al. have shown that particular gonococcal LPSs have carbohydrate structures that are analogous to human erythrocyte glycolipids, and that these two structures cross-react immunologically (83).

The antigenic variation of gonococcal LPS structures was seen on passage in vitro (81). This variability was also demonstrated in vivo during a human challenge study in which the strains isolated from the infected patient expressed different LPS antigenic types from the challenge strain (84).

An LPS-based vaccine would necessitate the detoxification of the endotoxic-producing properties of LPS. In addition, given the antigenic diversity of the LPS, a constant oligosaccharide portion or a type correlated with virulence would have to be identified. The immunogenicity of this molecule in humans and its apparent role in pathogenesis designate it as another attractive vaccine candidate (77).

H.8 Epitope

An epitope contained on two different neisserial lipoproteins that binds to a specific monoclonal antibody is called H.8. The epitope itself appears to be conserved and stable (85). Following its identification in 1984, H.8 gained wide attention because of its presence on pathogenic *Neisseria (N. gonorrhoeae, N. meningitidis)*, but absence on commensal *Neisseria* (85).

The two lipoproteins that contain the H.8 epitope are the lipid-modified azurin (Laz) and the H.8 outer membrane protein (Lip) (86).

The lipid-modified azurin is present in both pathogenic and commensal *Neisseria* (86). It is not reactive with the H.8 monoclonal antibody on Western blots. Like other azurin proteins, this lipid-modified azurin may function in electron transport during bacterial respiration. Its role, if any, in pathogenesis is not known.

The H.8 outer membrane protein is also a lipoprotein, but it is present on pathogenic *Neisseria* only. Its apparent molecular mass varies from 22 to 30 kDa among strains (87). This protein is alanine- and proline-rich and does not stain with Coomassie blue after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The H.8 outer membrane lipoprotein has been extracted and purified from neisserial organisms (87, 88), and the gene has been cloned and sequenced (89). The protein consists of a repeating heptapeptide. Differences in the number of repeating units may account for the difference in apparent molecular weight among strains. The function of Lip in the outer membrane is unknown, but it is believed to serve in a structural role.

The H.8 epitope appears to be surface-ex-

posed on viable gonococci, but electron microscopy studies with gold-labeled monoclonal antibodies have provided conflicting results (85, 86, 90).

Patients develop serum antibodies to the H.8 epitope after local genital infections, salpingitis, or disseminated gonorrhea (50, 51, 91). One patient was found to have H.8 antibody in seminal plasma (50). However, H.8 antibodies have also been found in the sera of patients without a prior history of gonorrhea. One study showed that the acquisition of *N. meningitidis* throat carriage can result in the development of H.8 antibody in the serum (50). In several studies, patients developed local gonococcal infections despite the presence of H.8 antibody in the serum (50, 66).

Most monoclonal antibodies specific for H.8 lack bactericidal activity and fail to protect against meningococcal infection in animal models (92). However, one H.8 monoclonal antibody has been shown to be bactericidal and opsonic for some gonococci (86). Affinity-purified human antibodies specific for H.8 were found to lack bactericidal activity for meningococci (93).

The value of the H.8 epitope as a gonococcal vaccine candidate is unclear. The apparent surface-exposure, stability, and association with pathogenic *Neisseria* make it worthy of pursuit; however, the lack of a known role in pathogenesis and the inability of serum antibody to prevent infection weigh against it.

IgA Protease

Immunoglobulin A represents a major host defense system against microbial pathogens that come in contact with mucosal surfaces (94). However, many bacteria, such as *N. gonorrhoeae*, *N. meningitidis*, *H. influenzae*, and *Streptococcus pneumoniae*, are known to produce a protease that cleaves human IgA1 at the hinge region (94). These IgA1 proteases are thought to act as a mechanism for bacteria to evade specific mucosal immunity. The construction of IgA1 protease-negative mutants of *N. gonorrhoeae* provides a valuable tool to investigate the significance of this enzyme in the pathogenesis of gonorrhea (95).

The gene encoding the gonococcal IgA1 protease has been cloned. Significant homology with IgA1 protease genes of other species was also found (96). Patients with local gonococcal infections, salpingitis, and disseminated gonorrhea infrequently produce antibody to IgA1 protease in their sera (97). Patients with meningococcal disease or meningococcal carriage more commonly produce specific antibody to the enzyme (97). This antibody is cross-reactive with IgA1 protease from *N. gonorrhoeae* and inhibits the protease activity of the enzyme (97).

The IgA protease presents an attactive vaccine candidate if its biological role in disease is validated. As part of a multicomponent vaccine, antibody raised to this enzyme may well allow IgAs of other specificities to be more efficacious in attacking the organism.

Major Iron-Regulated Protein

Neisseria gonorrhoeae expresses several proteins under iron-limited conditions (98). These proteins may be involved in iron uptake by the bacteria and therefore may have a function in pathogenesis. In contrast to many gram-negative bacteria that produce soluble siderophores, the gonococcus must obtain iron directly from specific iron-binding proteins (lactoferrin and transferrin) of the host (99).

The major iron-regulated protein (MIRP) is a 37-kDa protein and appears to be common among all gonococci and meningococci (100). Morse et al. have recently described its purification and characterization (101). Antibodies to MIRP have been detected in patients with disease, indicating that it is expressed in vivo (102). This protein is reported to bind iron from transferrin (101).

The apparent immunogenicity and conserved nature of the MIRP makes it an attractive vaccine candidate. Other proteins that mediate iron acquisition also deserve attention. Interference with iron utilization could potentially alter the course of disease.

Other Gonococcal Antigens

Anaerobic or aerobic growth conditions induce strains to selectively express a variety of membrane proteins. Other conditions of environmental stress result in the expression of another class of proteins called *stress proteins*. The role of these proteins in organism survival or pathogenesis is not known. Yet it is interesting to note the presence of antibodies to an anerobically induced protein in women with PID, thus suggesting its immunogenicity and expression in vivo (103).

Gonococcal outer membrane protein–macromolecular complex is a surface-exposed homopolymer. It is antigenically conserved and constitutes about 10% of the outer membrane protein (104). Antibodies raised to this antigen in animals exhibit bactericidal activ-

ity (104). Little is known of its function or role in disease.

Numerous other proteins, as yet unnamed and uncharacterized, appear in SDS-PAGE of gonococcal lysates. Some of these proteins appear to elicit a serum antibody response to infection (50, 51). Work is just beginning to evaluate their importance.

GENERATION OF MUCOSAL IMMUNITY

The presence and duration of genital mucosal immunity is an issue of critical importance for the development of a gonococcal vaccine. A parenteral injection with purified pili can induce in the genital tract antibodies that inhibit attachment of gonococci to epithelial cells in vitro (25, 105). In addition, studies in women have demonstrated high concentrations of antigonococcal IgA following infection (106, 107).

Humans may possess a "common" mucosal immune system in which antigenic presentation at one mucosal surface can lead to trafficking of secretory IgA–producing cells to other mucosal locations (108). Perhaps oral or intestinal immunization will lead to a genital immune response, since secretory IgA (S-IgA) is the major immunoglobulin of mucosal surfaces and is produced by plasma cells in the lamina propria (109). Exposure of an antigen to the Peyer's patches in the intestine can stimulate T cells of various types, and precursor IgA B cells, resulting in an IgA response at distant mucosal sites, such as the genital tract (108).

Examples of this phenomenon have been seen in oral immunization with *Streptococcus mutans* that resulted in significant S-IgA response in the saliva and tears (110). For *Neisseria*, a protein I vaccine administered in the intestines of rats induced antibody in lymphoid organs (111).

VACCINE PROSPECTS AND RESEARCH DIRECTIONS

Gonorrhea continues as an epidemic disease with serious complications, especially for the female. The current strategy of selective screening, contact tracing, education, and improved clinical care has served only to contain the epidemic in developed countries. Lacking resources for implementation of these strategies, less-developed countries have even greater problems.

Despite effective antibiotic treatment, the pool of minimally symptomatic and asymptomatic carriers serves as a reservoir for transmission. Upon clinical presentation, many women already have PID. While the infection is treatable, the sequelae remain. A strategy to prevent infection will have widespread impact, and a vaccine is highly desirable.

The evolution of antibiotic resistance in the gonococcus hastens the quest. Parenteral antibiotic injections for treatment are again needed in many regions, and close vigilance of susceptibility patterns is mandated.

The development of an effective gonococcal vaccine will be a formidable task. Many individuals acquire gonococcal infections repeatedly, implying the absence of or short-lived immunity. In the preantibiotic era, untreated infections persisted for months, implying markedly delayed, if any, disease-induced immunity. In part, this may be due to the usual confinement of infection to the local mucosa. Antigen processing at the genital mucosal surface is problematic. Moreover, antibody presence, particularly at the critical time of organism arrival, would be expected to be quantitatively minimal.

Gonococci also seemed equipped with an outstanding arsenal to avoid the host's immune defenses. The capacity for on–off switching that controls antigen expression (pili, protein II), combined with the capacity for antigenic variability (pili, protein II, LPS) is staggering. Another antigen, protein III, may actually serve as a target for cross-reactive antibodies and block access of specific functional antibodies. The suggestion of a shared epitope between LPS and the erythrocyte membrane raises the possibility of antigenic mimicry, a mechanism to avoid antibody production. Finally IgA protease may serve to destroy any secretory antibodies that arrive for defense.

Add to this the lack of protection of whole-cell and purified pili vaccines in human field trials, and the skepticism expressed by some concerning the development of an effective vaccine appears well founded.

But there are reasons for persistence and optimism. Older studies demonstrated a correlation between a prior gonococcal infection and relative resistance to urethral challenge (112). Two studies now suggest the development of protein I–type or serovar-specific immunity after female genital infection. Most encouragingly, there was development of ho-

mologous protection to urethral challenge after parenteral immunization with purified pili.

The ideal vaccine would be one that prevents colonization and local infection. Given the uniquely human reservoir of *N. gonorrhoeae*, such a vaccine may eventually lead to eradication of the organism. If prevention of local infection is not possible, an alternative approach would be a vaccine that limits extension of infection and prevents PID. Such a vaccine would have a profound impact on the morbidity of gonorrhea.

The evaluation of vaccine candidates must proceed cautiously. Should a vaccine allow or predispose to asymptomatic carriage, it might have a deleterious public health effect. Increasing the reservoir of carriers would predictably result in a higher incidence of gonococcal disease, including PID, among the unimmunized.

A successful vaccine will likely be one that induces antibodies that simultaneously attack the organism at several stages of pathogenesis. One might envision the success of vaccine-induced antibodies that allow human IgA survival, promote opsonization and bacteriolysis, and yet block organism adherence, iron usage, and eukaryotic membrane integration.

Appropriate models to evaluate pathogenesis and immunity are a problematic area. Numerous animal models have been employed, but only the chimpanzee urethritis model bears any resemblance to human disease. The availability, difficulty, and cost of these animals have curtailed the use of this model. Other animal models such as the guinea pig chamber and chick embryo are also in use, but their relevance to human disease is unknown.

Tissue and organ cultures are in selective use. Much about pathogenic mechanisms has been learned from the fallopian tube system.

Ultimately, the human will serve for the final validation of a successful vaccine. Human challenge studies have been of considerable value in the past for investigations on pathogenesis and for vaccine testing. The model is safe, and the experimental infection closely resembles naturally acquired disease. More studies are needed in this model to understand the contribution of specific gonococcal components to human disease and to investigate the human immune response. Ethical considerations limit this model to male urethritis only, so the evaluation of vaccines that might prevent pelvic inflammatory disease will have to take place in the field.

Ongoing and needed investigations that have the greatest bearing on vaccine development lie in the following three areas:

1. Studies on the molecular basis of pathogenesis that increase our understanding of the critical steps leading from organism exposure to infection are essential. Particular emphasis should be placed on the contribution of individual antigens to this process. Identification of gonococci–host cell interactions, as well as host cell receptors, are a critical phase of these studies.
2. Studies on the extent of antigenic variation, the mechanisms for its control, and the contribution of the host environment and immune response will allow a realistic assessment of potential vaccine candidates. The discovery of common, stable, and functional epitopes in this sea of variability holds the brightest hope for an effective vaccine.
3. Studies on the local immune response in the human genital tract, methods to enhance it, and the value of specific antibodies in protection are vital to our continued progress. Investigations should continue on local immunization procedures.

Many new technologies are now widely available to aid in these investigations. Gene cloning, DNA and protein sequencing, protein structure studies, synthetic peptides, new adjuvants, epitope mapping, the polymerase chain reaction, and the construction of defined mutants can now be brought to bear. It will likely require the close collaboration of many groups with diverse skills in order for us to be successful in the quest for an effective gonorrhea vaccine.

Note: The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense.

REFERENCES

1. Knapp JS: Historical perspectives and identification of *Neisseria* and related species. Clin Microbio-Rev 1988; 1:415–431.
2. Kampmeier RH: Identification of the gonococcus by Albert Neisser. Sex Transm Dis 1978; 5:71–72.
3. Kampmeier RH: Introduction of sulfonamide therapy for gonorrhea. Sex Transm Dis 1983; 10:81–84.

4. Miller CP, Scott WW, Moeller V: Studies on the action of penicillin: I. The rapidity of its effect on gonococci urethritis. JAMA 1944; 125:607–610.
5. Judson FN: Management of antibiotic-resistant *Neisseria gonorrhoeae*. Ann Intern Med 1989; 110:5–7.
6. Committee on Issues and Priorities for New Vaccine Development: Prospects for immunizing against *Neisseria gonorrhoeae*. In New Vaccine Development: Establishing Priorities. Washington, DC, National Academy Press, 1985, vol 1, pp 365–384.
7. Hook EW III, Holmes KK: Gonococcal infections. Ann Intern Med 1985; 102:229–243.
8. Petersen BH, Lee TJ, Synderman R, Brooks CF: *Neisseria meningitidis* and *Neisseria gonorrhoeae* bacteremia associated with C6, C7, C8 deficiency. Ann Intern Med 1979; 90:917–920.
9. Gotschlich EC: Gonorrhea. In Robbins JB, Schneerson R, Klein D, Sadoff J, Hardegree MC (eds): Bacterial Vaccines. New York, Academic Press, 1984; pp 353–371.
10. Ward ME, Robertson JN, Englefield PM, Watt PJ: Gonococcal infection: Invasion of the mucosal surfaces of the genital tract. In Schlessinger D. (ed): Microbiology—1975 Washington, DC, American Society for Microbioloy, 1975, pp 188–199.
11. McGee ZA, Horn RG: Phagocytosis of gonococci by nonprofessional phagocytic cells. In Schlessinger D. (ed): Microbiology–1979 Washington, DC, American Society of Microbiology, 1979, pp 158–161.
12. Greenberg L, Diena FA, Ashton FA, Wallace R, Kenney CP, Znamirowski R, Ferrari H, Atkinson J: Gonococcal vaccine studies in Inuvik. Can J Public Health 1974; 65:29–33.
13. Brinton CC, Wood SW, Brown A, Labik AM, Bryan JR, Lee SW, Polen SE, Tramont EC, Sadoff J, Zollinger W: The development of a neisserial pilus vaccine for gonorrhea and meningococcal meningitis. In Robbins JB, Hill JC, Sadoff JC (eds): Seminars in Infectious Disease. New York, Thieme-Stratton, 1982, vol 4: Bacterial Vaccines, pp 140–159.
14. Tramont EC, Boslego JW, Chung R, McChesney D, Ciak J, Sadoff J, Piziak M, Brinton CC, Wood S, Bryan J: Parenteral gonococcal pilus vaccine. In Schoolnik GK, Brooks GF, Falkow S, Frasch CE, Knapp JS, McCutchan JA, Morse SA (eds): The Pathogenic Neisseriae. Washington, DC, American Society for Microbiology, 1985, pp 316–322.
15. Hagblom P, Segal E, Billyard E, So M: Intragenic recombination leads to pilus antigenic variation in *Neisseria gonorrhoeae*. Nature 1985; 315:156–158.
16. Hermodson MA, Chen KCS, Buchanan TM: *Neisseria* pili proteins: Amino terminal amino acid sequences and identification of an unusual amino acid. Biochemistry 1978; 17:442–445.
17. Sastry PA, Pearlstone JR, Smillie LB, Paranchych W: Amino acid sequence of pilin isolated from *Pseudomonas aeruginosa* PAK. FEBS Lett 1983; 151:253–256.
18. Froholm LO, Sleten K: Purification and N-terminal amino acid sequence of a fimbrial protein from *Moraxella nonliquefaciens*. FEBS Lett 1977; 73:29–32.
19. McKern NM, O'Donnell IJ, Inolis AS, Stewart DJ, Clark BL: Amino acid sequence of pilin from *Bacteroides nodosus*, the causative organism of ovine footrot. FEBS Lett 1983; 164:149–153.
20. Kellogg DS Jr, Peacock WL Jr, Deacon WE, Brown L, Pickle CI: *Neisseria gonorrhoeae*: I. Virulence genetically linked to colonial variation. J Bacteriol 1963; 85:1274–1279.
21. Swanson J, Kraus SJ, Gotschlich EC: Studies on gonococcus infection: I. Pili and zones of adhesion: Their relationship to gonococcal growth patterns. J Exp Med 1971; 134:886–906.
22. Woods ML, McGee ZA: Molecular mechanisms of pathogenicity of gonococcal salpingitis. Drugs 1986; 3(suppl 2):1–6.
23. Heckels JE: Molecular studies on the pathogenesis of gonorrhoea. J Med Microbiol 1984; 18:293–306.
24. Blake MS, Swanson J: Studies on gonococcus infection: IX. In vitro decreased association of piliated gonococci with mouse peritoneal macrophages. Infect Immun 1975; 11:1402–1404.
25. Brinton CC, Bryan J, Dillon J, Guerina N, Jacobson LJ, Labik A, Lee S, Levine A, Lim S, McMichael J, Polen S, Rogers K, To ACC, To SCM: Uses of pili in gonorrhea control: Role of bacterial pili in disease, purification and properties of gonococcal pili, and progress in the development of a gonococcal pilus vaccine for gonorrhea. In Brooks GF, Gotschlich EC, Holmes KK, Sawyer WD, Young FE (eds): Immunobiology of *Neisseria gonorrhoeae*. Washington, DC, American Society for Microbiology, 1978, pp 155–178.
26. Swanson J, Robbins K, Barrera O, Corwin D, Boslego J, Ciak J, Blake M, Koomey MJ: Gonococcal pilin variants in experimental gonorrhea. J Exp Med 1987; 165:1344–1357.
27. Bergstrom S, Robbins K, Koomey JM, Swanson J: Piliation control mechanisms in *Neisseria gonorrhoeae*. Proc Natl Acad Sci USA 1986; 83:3890–3894.
28. Meyer TF, Haas R: Phase and antigenic variation by DNA rearrangements in procaryotes. Symp Soc Gen Microbiol 1988; 43:193–219.
29. Meyer TF, Mlawer N, So M: Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. Cell 1982; 30:45–52.
30. Haas R, Meyer
pilus genes in
dence for ge
44:107–115.
31. Seifert HS, A
model for *Neis*
tion. Vaccine I
32. Rothbard JB,
NNH, Schoolni
corresponding
gonococcal pi
Proc Natl Aca
33. Getzoff ED, F
JA: Understar
genicity of g
1988; 10(supp
34. Muir L, Strug
appear to be a
gonorrhoeae
1747.
35. Lindberg F, I
S: Localizatio
tein adhesion
Nature 1987;
36. Stromberg N,
So M, Karlsso
hydrate struc
tors for *Neis*
Acad Sci USA
37. Johnston KH
The serologi
gonorrhoeae:
brane comple
specificity. J
758.
38. Blake MS, C
immunogeni
seria surface
Bacterial Me
York, John
399.
39. Judd RC: St
tein I and p
seria gonor
37:632–641
40. Carbonetti
cloning and
tural gene
membrane
Natl Acad S
41. Gotschlich
M: Porin p
Cloning an
Sci USA 19
42. Joiner KA,
Monoclona
gonococcal
tivity. J Im
43. Tamm MR
Holmes KI
RC: Serol
gonorrhoe
fect Immu
44. Brunham I

30. Haas R, Meyer TF: The repertoire of silent pilus genes in *Neisseria gonorrhoeae*: Evidence for gene conversion. Cell 1986; 44:107–115.
31. Seifert HS, Ajioka R, So M: Alternative model for *Neisseria gonorrhoeae* pilin variation. Vaccine 1988; 6:107–109.
32. Rothbard JB, Fernandez R, Wang L, Teng NNH, Schoolnik GK: Antibodies to peptides corresponding to a conserved sequence of gonococcal pilins block bacterial adhesion. Proc Natl Acad Sci USA 1985; 82:915–919.
33. Getzoff ED, Parge HE, McRee DE, Tainer JA: Understanding the structure and antigenicity of gonococcal pili. Rev Infect Dis 1988; 10(suppl 2):S296–S299.
34. Muir L, Strugnell R, Davies J: Proteins that appear to be associated with pili in *Neisseria gonorrhoeae*. Infect Immun 1988; 56:1743–1747.
35. Lindberg F, Lund B, Johansson L, Normark S: Localization of the receptor-binding protein adhesion at the tip of the bacterial pilus. Nature 1987; 328:84–87.
36. Stromberg N, Deal C, Nyberg G, Normark S, So M, Karlsson KA: Identification of carbohydrate structures that are possible receptors for *Neisseria gonorrhoeae*. Proc Natl Acad Sci USA 1988; 85:4902–4906.
37. Johnston KH, Holmes KK, Gotschlich EC: The serological classification of *Neisseria gonorrhoeae*: I. Isolation of the outer membrane complex responsible for the serotypic specificity. J Exp Med 1976; 143:741–758.
38. Blake MS, Gotschlich EC: Functional and immunogenic properties of pathogenic *Neisseria* surface proteins. In Inouye M (ed): *Bacterial Membranes as Model Systems*. New York, John Wiley & Sons, 1987, pp 377–399.
39. Judd RC: Surface peptide mapping of protein I and protein III of four stains of *Neisseria gonorrhoeae*. Infect Immun 1982; 37:632–641.
40. Carbonetti NH, Sparling PF: Molecular cloning and characterization of the structural gene for protein I, the major outer membrane of *Neisseria gonorrhoeae*. Proc Natl Acad Sci USA 1987; 84:9084–9088.
41. Gotschlich EC, Seiff ME, Blake MS, Koomey M: Porin protein of *Neisseria gonorrhoeae*: Cloning and gene structure. Proc Natl Acad Sci USA 1987; 84:8135–8139.
42. Joiner KA, Warren KA, Tam M, Frank M: Monoclonal antibodies directed against gonococcal protein I vary in bactericidal activity. J Immunol 1985; 134:3411–3419.
43. Tamm MR, Buchanan TM, Sandstrom EG, Holmes KK, Knapp JS, Siadak AW, Nowinski RC: Serological classification of *Neisseria gonorrhoeae* with monoclonal antibodies. Infect Immun 1982; 36:1042–1053.
44. Brunham RC, Plummer F, Slaney L, Rand F, DeWitt W: Correlation of auxotype and protein I type with expression of disease due to *Neisseria gonorrhoeae*. J Infect Dis 1985; 152:339–343.
45. Rice RJ, Biddle JW, JeanLouise YA, DeWitt WE, Blount JH, Morse SA: Chromosomally mediated resistance in *Neisseria gonorrhoeae* in the United States: Results of surveillance and reporting, 1983–1984. J Infect Dis 1986; 153:340–345.
46. O'Brien JP, Goldenberg DL, Rice PA: Disseminated gonococcal infection: A prospective analysis of 49 patients and a review of pathophysiology and immune mechanisms. Medicine 1983; 62:395–406.
47. Knapp JS, Holmes KK, Bonin P, Hook EW III: Epidemiology of gonorrhea: Distribution and temporal changes in auxotype/serovar classes of *Neisseria gonorrhoeae*. Sex Transm Dis 1987; 14:26–32.
48. Knapp JS, Tam MR, Nowinski RC, Holmes KK, Sandstrom EG: Serological classification of *Neisseria gonorrhoeae* with use of monoclonal antibodies to gonococcal outer membrane protein: I. J Infect Dis 1984; 150:44–48.
49. Knapp JS, Zenilman JM, Biddle JW, Perkins CH, DeWitt WE, Thomas ML, Johnson SR, Morse SA: Frequency and distribution in the United States of strains of *Neisseria gonorrhoeae* with plasmid-mediated high-level resistance to tetracycline. J Infect Dis 1987; 155:819–822.
50. Hicks CB, Boslego JW, Brandt B: Evidence of serum antibodies to *Neisseria gonorrhoeae* before gonococcal infection. J Infect Dis 1987; 155:1276–1281.
51. Lammell CJ, Sweet RL, Rice PA, Knapp JS, Schoolnik GK, Heilbron DC, Brooks GF: Antibody-antigen specificity in the immune response to infection with *Neisseria gonorrhoeae*. J Infect Dis 1985; 152:990–1001.
52. Fohn MJ, Mietzner TA, Hubbard TW, Morse SA, Hook EW III: Human immunoglobulin G antibody response to the major gonococcal iron-regulated protein. Infect Immun 1987; 55:3065–3069.
53. Hook EW III, Olsen DA, Buchanan TM: Analysis of antigen specificity of the human serum immunoglobulin G immune response to complicated gonococcal infection. Infect Immun 1984; 43:706–709.
54. Sarafian SK, Tam MR, Morse SA: Gonococcal protein I-specific opsonic IgG in normal human serum. J Infect Dis 1983; 148:1025–1032.
55. Heckels JE, Virji M, Zak K, Fletcher JN: Immunobiology of gonococcal outer membrane protein: I. Antonie Van Leeuwenhoek J Microbiol 1987; 53:461–464.
56. Buchanan T, Eschenbach D, Knapp J, Holmes K: Gonococcal salpingitis is less likely to recur with *Neisseria gonorrhoeae* of the same principal outer membrane protein

antigenic type. Am J Obstet Gynecol 1981; 138:978–980.

57. Plummer FA, Simonsen JN, Chubb H, Slaney L, Kimata J, Bosire M, Ndinya-Achola JO, Ngugi EN: Epidemiologic evidence for the development of serovar-specific immunity after gonococcal infection. J Clin Invest 1989; 83:1472–1476.
58. Virji M, Zak K, Heckels JE: Monoclonal antibodies to gonococcal outer membrane protein IB: Use in investigation of the potential protective effect of antibodies directed against conserved and type-specific epitopes. J Gen Microbiol 1986; 132:1621–1629.
59. Sparling PF, Cannon JG, So M: Phase and antigenic variation of pili and outer membrane protein II of *Neisseria gonorrhoeae*. J Infect Dis 1986; 153:196–201
60. Swanson J: Colony opacity and protein II compositions of gonococci. Infect Immun 1982; 37:359–368.
61. Swanson J: Studies on gonococcus infection: XII. Colony color and opacity variants of gonococci. Infect Immun 1978; 19:320–331.
62. Robinson EN, Clemens CM, McGee ZA, Cannon JG: Immunoelectron microscopic localization of outer membrane protein II on the surface of *Neisseria gonorrhoeae*. Infect Immun 1988; 56:1003–1006.
63. Fischer SH, Rest RF: Gonococci processing only certain P.II outer membrane proteins interact with human neutrophils. Infect Immun 1988; 56:1574–1579.
64. Lambden PR, Heckels JE, James LT, Watt PJ: Variations in surface protein composition associated with virulence properties in opacity types of *Neisseria gonorrhoeae*. J Gen Microbiol 1979; 114:305–312.
65. James JF, Swanson J: Studies on gonococcus infection: XIII. Occurrence of color/opacity colonial variants in clinical cultures. Infect Immun 1978; 19:332–340.
66. Brooks GF, Lammel CJ: Humoral immune response to gonococcal infections. Clin Microbiol Rev 1989; 2(suppl):S5–S10.
67. Swanson J, Barrera O, Sola J, Boslego J: Expression of outer membrane protein II by gonococci in experimental gonorrhea. J Exp Med 1988; 168:2121–2129.
68. Swanson J, Mayer LW, Tam MR: Antigenicity of *Neisseria gonorrhoeae* outer membrane protein(s) III detected by immunoprecipitation and Western blot transfer with a monoclonal antibody. Infect Immun 1982; 38:668–672.
69. Blake MS, Wetzler LM, Gotschlich C, Rice PA: Protein III: Structure, function, and genetics. Clin Microbiol Rev 1989; 2(suppl):S60–S63.
70. Judd RC: ^{125}I-peptide mapping of protein III isolated from four strains of *Neisseria gonorrhoeae*. Infect Immun 1982; 37:622–631.
71. Blake MS, Lytton EJ, Seiff ME, Gotschlich EC: Studies on gonococcal protein III. In Horwitz MA (ed): Bacteria-Host Cell Interaction. New York, Alan R Liss, 1988, pp 85–97.
72. Joiner KA, Warren KA, Frank MM, Rice PA: Blocking immunoglobulin G enhances complement consumption and deposition on *Neisseria gonorrhoeae*. In Schoolnik GK, Brooks GF, Falkow S, Frasch CE, Knapp JS, McCutchan JA, Morse SA (eds): The Pathogenic Neisseriae. Washington, DC, American Society of Microbiology, 1985, pp 431–434.
73. Rice PA, Tam MR, Blake MS: Immunoglobulin G antibodies in normal human serum directed against protein III block killing of serum-resistant *Neisseria gonorrhoeae* by immune human serum. In Schoolnik GK, Brooks GF, Falkow S, Frasch CE, Knapp JS, McCutchan JA, Morse SA (eds): The Pathogenic Neisseriae. Washington, DC, American Society for Microbiology, 1985, pp 427–430.
74. Joiner KA, Scales R, Warren KA, Frank MM, Rice PA: Mechanism of action of blocking immunoglobulin G for *Neisseria gonorrhoeae*. J Clin Invest 1985; 76:1765–1772.
75. Rice PA, Vayo HE, Tam MR, Blake MS: Immunoglobulin G antibodies directed against protein III block killing of serum-resistant *Neisseria gonorrhoeae* by immune serum. J Exp Med 1986; 164:1735–1748.
76. Gregg CR, Johnson AP, Taylor-Robinson D, Melly MA, McGee AZ: Host species-specific damage to oviduct mucosa by *Neisseria gonorrhoeae* lipopolysaccharide. Infect Immun 1981; 34:1056–1958.
77. Apicella M, Westerink M, Morse S, Schneider H, Rice P, Griffiss JM: Bactericidal antibody response of normal human serum to the lipooligosaccharide of *Neisseria gonorrhoeae*. J Infect Dis 1986; 153:520–526.
78. Griffiss JM, Schneider H, O'Brien JP: Lysis of *Neisseria gonorrhoeae* initiated by binding of normal human immunoglobulin into an hexosamine-containing lipooligosaccharide epitope is augmented by strain permissive feedback through the alternate pathway of complement activation. In Schoolnik GK, Brooks GF, Falkow S, Frasch CE, Knapp JS (eds): The Pathogenic Neisseriae. Washington, DC, American Society for Microbiology, 1985, pp 456–461.
79. Schneider H, Griffiss JM, Mandrell RE, Jarvis GA: Elaboration of a 3.6-kilodalton lipooligosaccharide, antibody against which is absent from human sera, is associated with serum resistance of *Neisseria gonorrhoeae*. Infect Immun 1985; 50:672–677.
80. Griffiss JM, Schneider H, Mandrell RE, Tamasaki R, Jarvis GA, Kim JJ, Gibson BW, Hamadeh R, Apicella MA: Lipooligosaccharides: The principal glycolipids of the neisserial outer membrane. Rev Inf Dis 1988; 10(suppl 2):S287–S295.
81. Schneider H
RC Jr, Ham
geneity of
pression wi
vidual strai
Neisseria m
45:544–54
82. Gibson BW
SJ, Burling
H, Griffiss
of the oligo
charides o
orrhoeae.
86:17–21
83. Mandrell F
ligosaccha
rhoeae an
ponents t
to precur
gens: Car
the mous
ognize cr
human e
168:107–
84. Schneide
Hamma
Neisseria
disease a
brane gl
In: Prog
Eighth I
crobial
ington D
ogy, 19
85. Cannon
P: Mon
outer m
genic N
pathog
1985;
86. Cannon
pathog
epitop
outer
1989;
87. Bhatta
ger W
H.8 a
gitidis
88. Stritt
preli
the
1986
89. Gots
M, I
gene
III
199
90. Hitc
Tes
tige
stra
usu
act
20

81. Schneider H, Hale TL, Zollinger WD, Seid RC Jr, Hammack CA, Griffiss JMcL: Heterogeneity of molecular size and antigenic expression within lipooligosaccharides of individual strains of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Infect Immun 1984; 45:544–549.

82. Gibson BW, Webb JW, Yamasaki R, Fisher SJ, Burlingame AL, Mandrell RE, Schneider H, Griffiss JM: Structure and heterogeneity of the oligosaccharides from the lipopolysaccharides of a pyocinresistant *Neisseria gonorrhoeae*. Proc Natl Acad Sci USA 1989; 86:17–21.

83. Mandrell RE, Griffiss JM, Macher BA: Lipooligosaccharides (LOS) of *Neisseria gonorrhoeae* and *Neisseria meningitidis* have components that are immunochemically similar to precursors of human blood group antigens: Carbohydrate sequence specificity of the mouse monoclonal antibodies that recognize crossreacting antigens on LOS and human erythrocytes. J Exp Med 1988; 168:107–126.

84. Schneider H, Boslego JW, Apicella MA, Hammack CA, Mandrell RE, Griffiss JM: *Neisseria gonorrhoeae* that cause urethral disease are clonal variants that share membrane glycolipids with human erythrocytes. In: Program and Abstracts of the Twenty-Eighth Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington DC, American Society for Microbiology, 1988, p 361, Abstract 1407.

85. Cannon JG, Black W, Nachamkin I, Stewart P: Monoclonal antibody that recognizes an outer membrane antigen common to pathogenic *Neisseria* species but not to most nonpathogenic *Neisseria* species. Infect Immun 1985; 43:994–999.

86. Cannon JG: Conserved lipoproteins of pathogenic *Neisseria* species bearing the H.8 epitope: Lipid-modified azurin and H.8 outer membrane protein. Clin Microbio Rev 1989; 2(suppl):S1–S4.

87. Bhattacharjee AK, Moran EE, Ray JS, Zollinger WD: Purification and characterization of H.8 antigen from group B *Neisseria meningitidis*. Infect Immun 1988; 56:773–778.

88. Strittmatter W, Hitchcock PJ: Isolation and preliminary biochemical characterization of the gonococcal H.8 antigen. J Exp Med 1986; 164:2038–2048.

89. Gotschlich EC, Blake MS, Koomey JM, Seiff M, Derman A: Cloning of the structural genes of three H.8 antigens and of protein III of *Neisseria gonorrhoeae*. J Exp Med 1986; 164:868–881.

90. Hitchcock P, Hayes S, Mayer L, Schafer W, Tessier S: Analysis of the gonococcal H.8 antigen: Surface location, inter- and intrastrain electrophoretic heterogeneity and unusual two-dimensional electrophoretic characteristics. J Exp Med 1985; 162:2017–2034.

91. Black JR, Black WJ, Cannon JG: Neisserial antigen H.8 is immunogenic in patients with disseminated gonococcal and menigococcal infection. J Infect Dis 1985; 151:650–657.

92. Woods JP, Black JR, Barritt DS, Connell TD, Cannon JG: Resistance to meningococcemia apparently conferred by anti-H.8 monoclonal antibody is due to contaminating endotoxin and not to specific immunoprotection. Infect Immun 1987; 55:1927–1928.

93. Zollinger WD: Meningococcal Meningitis. In Cryz SJ Jr. (ed): Vaccines and Immunotherapy. New York, Pergamon Press, 1991, pp 113–126.

94. Plaut AG: Microbial IgA proteases. N Engl J Med 1978; 298:1459–1463.

95. Koomey JM, Gill RE, Falkow S: Genetic and biochemical analysis of gonococcal IgA1 protease: Cloning in *Escherichia coli* and construction of mutants of gonococci which fail to produce the activity. Proc Natl Acad Sci USA 1982; 79:7881–7885.

96. Koomey JM, Falkow S: Nucleotide sequence homology between the immunoglobulin A1 protease genes of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Haemophilus influenzae*. Infect Immun 1984; 43:101–107.

97. Lammel CJ, Blake MS, Zollinger WD, Hook EW III, Bolan GA, Brooks GF: Antibodies against IgA1 protease in neisserial infection. In: Program and Abstracts of the Twenty-Eighth Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington DC, American Society for Microbiology, 1988, p 144, Abstract 195.

98. Norqvist A, Davis J, Norlander L, Normark S: The effect of iron starvation on the outer membrane protein composition of *Neisseria gonorrhoeae*. FEMS Microbiol Lett 1978; 4:71–75.

99. West SEH, Sparling PF: Response of *Neisseria gonorrhoeae* to iron-limitation: Alterations in expression of membrane proteins without apparent siderophore production. Infect Immun 1985; 47:388–394.

100. Mietzner TA, Luginbuhl GH, Sandstrom E, Morse SA: Identification of an iron-regulated 37,000-dalton protein in the cell envelope of *Neisseria gonorrhoeae*. Infect Immun 1984; 45:410–416.

101. Morse SA, Chen CY, LeFaou A, Mietzner TA: A potential role for the major iron-regulated protein expressed by pathogenic *Neisseria* species. Rev Infect Dis 1988; 10(suppl 2):S306–S310.

102. Morse SA, Meitzner T, Schalla WO, Lammel CJ, Brooks GF: Serum and vaginal fluid antibodies against the major iron-regulated protein in women with gonococcal pelvic inflammatory disease or uncomplicated infection. In: Abstract Book: Fifth International Pathogenic Neisseria Conference. Noordwijkerhout, the Netherlands, 1986, Abstract V-127.

103. Clark VL, Knapp JS, Thompson S, Klimpel

Ronald W. Ellis, Ph.D.

29 New Technologies for Making Vaccines

The development of new techniques in molecular genetics has expanded the number of approaches that can be used for making vaccines. In some cases, established vaccines can be improved or their supply increased. In other cases, new vaccines can be developed that have not been feasible through the application of old technologies. In this regard, it is worth considering first the broad categories into which vaccines can be divided. "Live" vaccines are defined by the ability of the vaccine strain, i.e., of the virus, to replicate within the human host. Conversely, "killed" vaccines ("non-live" may be more accurate, even though most scientists use the term "killed") are unable to replicate or infect the host. Table 29–1 summarizes the salient features of these two categories of vaccines.

Live Vaccines

Live vaccines are attenuated with respect to their ability to cause disease, meaning that they are less likely to cause clinical illness than the natural disease-causing agent. By virtue of their ability to undergo limited replication in the host, such vaccines, typically viruses, often induce cell-mediated (T cell) immunity in addition to antibody-mediated (B cell) immunity. As a result of such a broad spectrum of immunity as well as re-exposures to the virus which silently boost immunity, protection following a single inoculation with a live attenuated vaccine often lasts a lifetime. However, the ability of the live vaccine to replicate can be detrimental; being genetically plastic, a replicating virus can revert to a more pathogenic form and cause adverse reactions in a vaccinee or a contact of a vaccinee. Sufficient data must be obtained in animal studies as well as in clinical studies to rule out the possibility of reversion.

A number of strategies have been employed for developing live viral vaccines that are attenuated, as summarized in Table 29–2.

Several of these approaches were possible before the development of modern techniques in recombinant DNA (rDNA) technology which enable the manipulation of viruses on the molecular level. These classic approaches, which utilize routine techniques in cell culture, include attenuation in cell culture, selection for temperature-sensitive or cold-adapted viruses, isolation of closely related viruses from other species and selection for reassorted viruses from the progeny of an infection by two parental viruses. (These strategies are discussed in greater detail elsewhere with respect to particular vaccines.)

The ability to alter directly the structure of viruses on the molecular level is enabling scientists to design attenuated vaccines rather than forcing them to rely upon phenotypic selection and upon chance to provide the only mechanisms for viral change. Through techniques of viral genetics and DNA sequence analysis, it is possible to identify those regions in the viral genome where alteration can contribute to the attenuation of viral pathogenicity. This rDNA technol-

Table 29–1. General Characteristic of Vaccines

"Live" Vaccines
Attenuated with respect to pathogenicity
Cell-mediated immunity in addition to humoral immunity
Longer-lasting protection
Tendency to reactogenicity
Ability to revert
"Killed" Vaccines
Nonreplicating
Noninfectious
Lower reactogenicity
Need for boosters
High purity

Table 29–2. Strategies for the Development of Attenuated Live Viral Vaccines

"Classic" Approaches
Modified by passage in cell culture
Variant viruses from other species
Temperature-selected mutants
Reassorted genomes
"Molecular" Approaches
DNA modification mutants
Recombinant viruses

ogy allows such regions to be altered or deleted and introduced into the genome of a wild-type virus, thus leading to the production of an attenuated virus. This approach is presented in the schematic in Figure 29–1.

The salient feature of this approach is the deliberate construction of an attenuated virus that is unlikely to revert to a more pathogenic form. This construction is made possible by deleting a portion of a key region of the genome in such a way that reversion is ruled out. This approach first was applied successfully by Kit and coworkers to the attenuation of pseudorabies virus, thus leading to the creation of a safer vaccine for the prevention of a severe disease in pigs.[1] This is the first genetically altered live vaccine that was licensed for use in any species. A related approach is being taken for poliovirus and is applicable to other vaccines for humans.[2]

A second approach is the genetic alteration of a live virus to function as a vector, *i.e.*, carrier, for other genes. This approach enables the recombinant virus to function as a vaccine for two or more infectious agents in a single inoculation. This technology first was applied to vaccinia virus.[3, 4] Prior to this application, wild-type vaccinia virus had been used for the worldwide eradication of smallpox and is the prime example of a variant virus from another species used as a vaccine for humans. A region of the genome of vaccinia virus was identified as nonessential for viral replication by the general approach outlined in Figure 29–1. Within a plasmid containing this nonessential region, a gene encoding a surface protein of another pathogen was inserted (Fig. 29–2). This recombinant plasmid was introduced together with wild-type virus into cells in culture, resulting in the creation of a recombinant virus that carries the foreign gene.

For insertion into a virus vector, a gene is selected that encodes an immunogen, usually a surface protein, of a virus or a microbial parasite. In order for this strategy to be effective, the presentation of this immunogen during the course of viral replication should result in a protective immune response directed to the antigen and, therefore, the pathogen. Recombinant vaccinia viruses have been derived that express immunogens for hepatitis B virus, herpes simplex virus, influenza virus, rabies virus, Epstein-Barr virus and respiratory syncytial virus. Some of these recombinant viruses have shown promise in animal studies. A similar approach has been taken with respect to the genetic engineering of two human herpesviruses as viral vectors, herpes simplex virus[5] and varicella-zoster virus.[6]

Table 29–3 outlines several points that are important to the safety and efficacy of such live recombinant vaccines. A nonessential (i.e., not required for viral replication) region for the insertion of a foreign gene often can be used that will result in the attenuation of viral pathogenicity.[7] Multiple foreign genes can be inserted into a single viral genome, resulting in an immune response against multiple pathogens.[8] The level of expression of the foreign protein should be high enough to elicit effective immunity. The parental (vector) virus should be tested extensively; its use as a vaccine should be free of side effects. In that regard, the use of the smallpox vaccine strain of vaccinia virus has raised concern with respect to the neurological and dermatological sequelae observed in small numbers of vac-

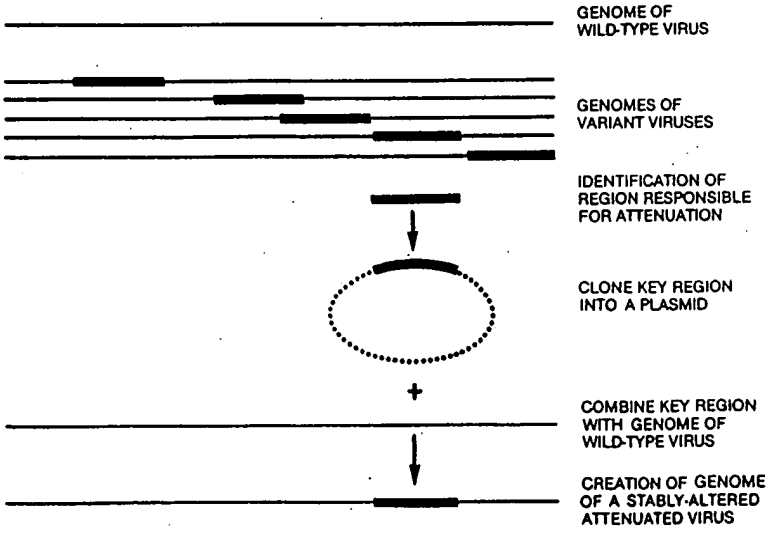


Figure 29–1. Attenuation of viruses using modern techniques in molecular biology.

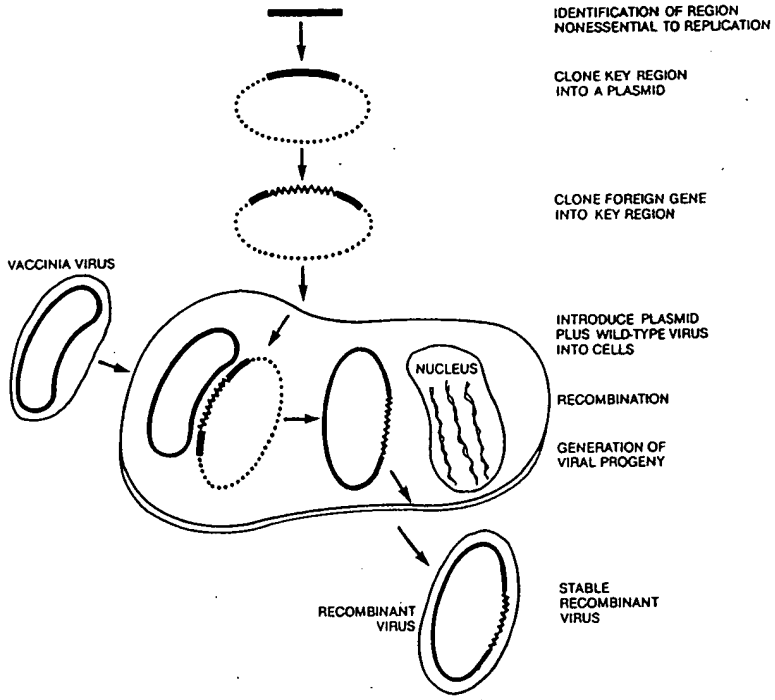


Figure 29–2. Creation of recombinant vaccinia viruses carrying genes that encode immunogens of other pathogens.

cinees. The host range or tissue tropism of the recombinant virus should not be altered significantly compared with that of the vector virus. The effects of viral infection upon the replication and structure of host cells should be studied closely. Since vaccinia virus encodes a protein with significant homology to transforming growth factor-α and to epidermal growth factor (EGF) and since the virus infects cells through the EGF receptor,[9] which is itself highly homologous to the *erb*-B oncogene, there is concern that infection with vaccinia virus may be mitogenic (stimulates growth or division of infected cells). Finally, while some recombinant vaccinia viruses have shown promise in preclinical testing in models of efficacy in animals, only clinical trials and testing of protective efficacy in humans, still awaited, will permit a complete assessment of the utility of such vaccines.

Table 29–3. Considerations in the Safety and Efficacy of Recombinant Live Vaccines

Safety
Extensive testing of parental virus
Stable attenuation of parental virus
Insertion point for the foreign gene
Host range of the recombinant virus
Biology of the cellular receptor for the virus
Efficacy
Multiple foreign genes in a single virus vaccine
Level of expression of foreign protein
Clinical testing

Killed Vaccines

In contrast to live vaccines, killed vaccines do not replicate in the host. Consequently, killed vaccines are often less efficient in the induction of cell-mediated immunity. In order to achieve complete and long-term protection, booster inoculations are required. Furthermore, the greater antigenic mass required for a killed vaccine to be effective, when compared with the antigenic mass for a live vaccine, raises issues of purity. Since they do not replicate, killed vaccines cannot revert to cause clinical disease. Several strategies have been used to develop killed vaccines, as summarized in Table 29–4.

The classic approaches, which employ techniques of biochemical purification and biophysical inactivation, include physical inactivation of whole viruses or bacteria, utilization of inactivated toxoids from bacteria, purification of mon-

Table 29–4. Strategies for the Development of Killed Vaccines

"Classic" Approaches
Killed whole pathogens
Toxoids from pathogens
Purified surface components
Conjugated surface components
"Molecular" Approaches
Recombinant-derived proteins
Synthetic peptides
Anti-idiotypic antibodies

omeric or aggregated surface components of viruses or bacteria and conjugation of surface components of bacteria to other molecules. (These strategies are discussed in greater detail elsewhere.)

The techniques of rDNA have revolutionized biomedical research. They make it possible to identify the gene encoding any protein of interest and to insert that gene into a host cell in such a way that the cell can produce large amounts of the particular protein (Fig. 29–3).

This technology is directly applicable to the development of vaccines. The key to the problem is the identification of that protein component of a virus or microbial pathogen that itself can elicit the production of protective antibodies, such antibodies having the capacity to neutralize infectivity and thus protect the host against attack by the pathogen. The protein then defines biochemical tools for research (e.g., antibodies and amino acid sequences), which are useful for the identification and cloning of the gene encoding that protein. Ultimately, the gene is placed into a host cell in a configuration that will result in synthesis by the host cell of large amounts of the particular immunogenic protein.

The initial application of rDNA technology to the development of vaccines for humans was for the vaccine to prevent infection by hepatitis B virus (HBV). A safe and effective vaccine, consisting of particles of the surface antigen of HBV (HBsAg) has been prepared from human plasma. In order to expand the available supply of vaccine, scientists turned to rDNA technology for vaccine production. The process was initiated by the identification of the gene encoding HBsAg and the insertion of that gene into various host cells. Recombinant yeast synthesize large amounts of particles of HBsAg that are morphologically (Fig. 29–4) and immunologically highly similar to the plasma-derived HBsAg.[10]

Recently, the yeast-derived HB vaccine produced by Merck, Sharp & Dohme became the first rDNA-derived vaccine of any type for humans ever to be licensed anywhere in the world. This prototype vaccine offers hope for the development of a new generation of vaccines, including ones for diseases such as malaria[11, 12] and leprosy[13] for which vaccines cannot be made using classic technologies. The development of recombinant vaccines ultimately may be facilitated by the application of new techniques for the enhancement of the immunogenicity of isolated proteins; one such technique is hydrophobic aggregation.[14] However, because of the biology of the disease or the nature of the immune response induced by the vaccine, it is important to realize that recombinant vaccines do not always provide the solution to the problem of prevention of an infectious disease.

There are a large number of host cells that can be utilized for the production of rDNA-derived proteins. The most common host cells have been bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*) and mammalian cells (Chinese hamster ovary, monkey kidney). Recently, scientists

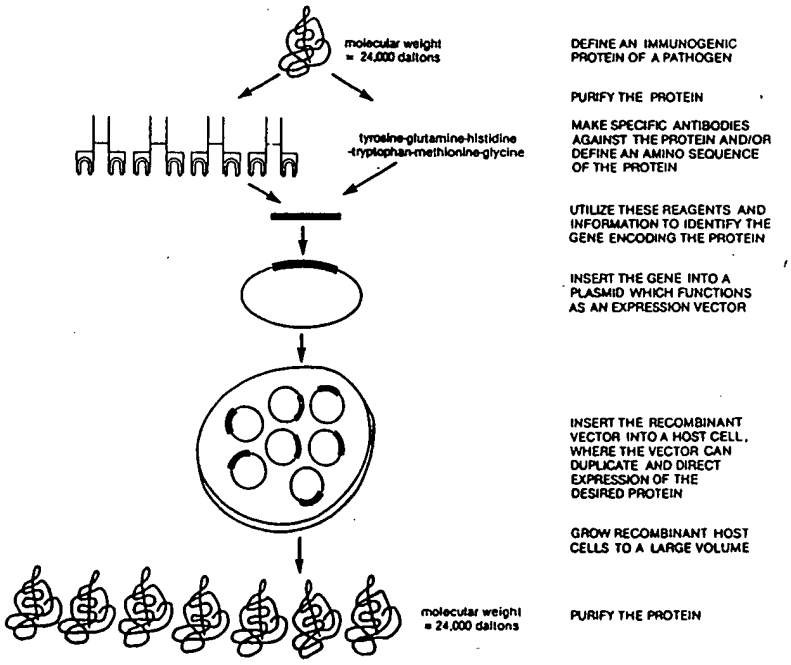


Figure 29–3. The use of recombinant DNA (rDNA) technology to express large amounts of a desired protein.

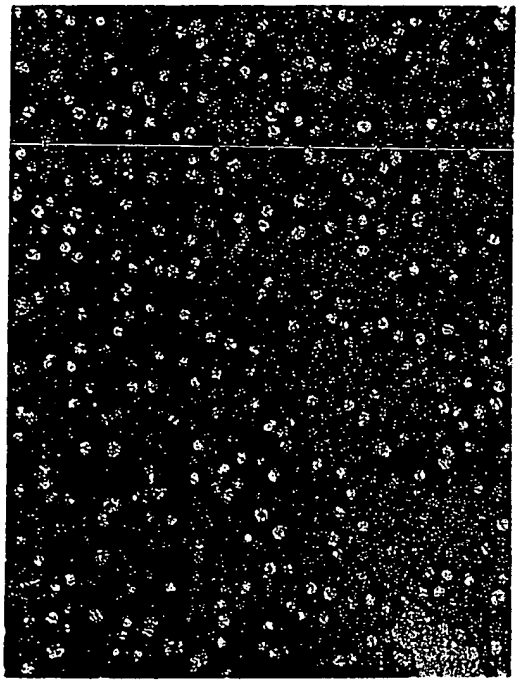


Figure 29–4. Electron micrograph of particles of HBsAg produced by recombinant yeast (165,000X). (Courtesy of B. Wolanski, Merck Sharp & Dohme Research Laboratories.)

have diversified to the use of other bacterial (*Bacillus subtilis*), fungal (*Aspergillus nidulans*) and higher eukaryotic (insert) cells. All these systems can be judged by a wide range of criteria relating to desirable traits of either the protein product or the host cell as well as to safety considerations (Table 29–5).

The most commonly employed expression systems can be evaluated relative to one another

Table 29–5. Expression Systems for rDNA-derived Proteins

Desirable Traits of the Product
High yields (commercial)
Stability of yield with scale-up of cells
Inducible expression
Secretion
Post-translational modifications (consistent with immunogenicity)
Glycosylation
Phosphorylation
Amidation
Carboxylation
Hydroxylation
Proteolytic processing
Desirable Traits of the Host Cells
Ease of scale-up
Consistency of performance
Lack of oncogenic elements
Rapid division
Safety Concerns
Heterologous protein contaminants
Biology of cell substrate
Residual DNA (oncogenesis)

*Scheme to take cultures from bench to large-scale fermentation or purification.

with respect to each of these criteria (Table 29–6). These criteria fall into three groups, which roughly discriminate between the microbial (yeast and bacteria) and mammalian expression systems as follows:

1. The microbial systems are more productive and consistent in overall performance than the mammalian ones.
2. Mammalian cells provide for post-translational modifications that often resemble more closely those in the viral agent than those provided by microbial cells.
3. With few exceptions, serially propagated

Table 29–6. Comparison of Commonly Used Expression Systems for rDNA

	E. coli (Bacteria)	*S. cerevisiae* (Yeast)	Chinese Hamster Ovary (Mammalian Cells)
Yield of product	+ + +	+ + +	+
Ease of scale-up	+ + +	+ + +	+
Stability of yield with scale-up	+ + +	+ + +	+
Inducible expression	+ + +	+ + +	+
Consistency of performance	+ + +	+ + +	+
Secretion	+	+ +	+ + +
Glycosylation	–	+ +	+ + +
Proteolytic processing	–	+ +	+ + +
Other modifications	–	+ +	+ + +
Biology of cell substrate	+ +	+ + +	+
Heterologous protein contaminants	+ +	+ +	+
Residual DNA	+ + +	+ + +	+

+ + + = most acceptable
+ + = acceptable
+ = least acceptable
– = absent

mammalian cells, unlike microbial cells, are considered "transformed," meaning that they are more susceptible to oncogenicity in experimental animals.

Furthermore, for expression of rDNA, mammalian cells often utilize genetic elements derived from oncogenic or latent viruses, while microbial cells do not utilize such elements. These perceived safety concerns must be addressed regarding the use of mammalian cells as an expression system for recombinant vaccines.

These relative evaluations represent generalizations from a large number of studies in the different systems and should be considered whenever an expression system is utilized. Nevertheless, each attempt at expression must be evaluated individually, and there are probably as many exceptions as there are rules in the "expression game"!

The use of synthetic peptides as vaccines involves the use of short segments of a protein molecule, rather than the entire molecule, as the immunogen. Some peptides are able to induce antibodies that can react with the whole protein as well as with the peptide per se.[15] The discovery process for formulating synthetic peptide antigens begins by defining the gene encoding the immunogenic protein (see Fig. 29–3), then branches off by exploiting the DNA sequence of the gene to define the amino acid sequence of the protein and to predict which regions of the protein might be immunogenic (Fig. 29–5). Once defined, peptides can be synthesized chemically[16] and formulated into synthetic vaccines.

This approach first was applied to the development of vaccines for humans by synthesizing portions of the HBsAg polypeptide.[17] In theory, the approach is technically versatile and lends itself to the production of well-defined vaccines. However, in practice, the approach has several shortcomings relative to the use of whole proteins. In general, the antibodies elicited by an intact protein crossreact more effectively with both the protein and the pathogen on which it resides than do antibodies elicited by a synthetic peptide. Furthermore, such antibodies bind with higher affinity and are present at a higher titer than are those elicited by the peptide. Thus, the duration of the immune response stimulated by a synthetic peptide is inferior to that stimulated by a whole protein. At minimum, a complete cocktail of synthetic peptides may be required as well as an improvement in methods for the enhancement of immunogenicity by covalent conjugation onto carrier proteins. It may be that synthetic peptides, however tailored, cannot mimic all the conformations assumed by the

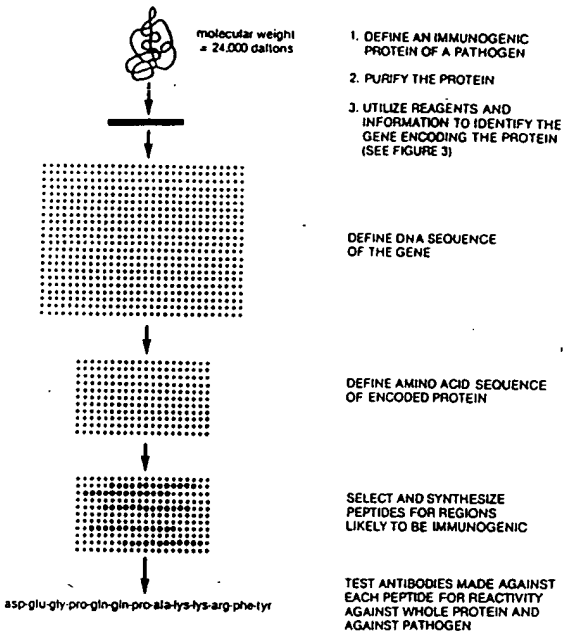


Figure 29–5. Defining immunogenic peptides from immunogenic proteins.

intact protein that are critical for immunogenicity. Furthermore, immunogens often have complex chemical structures, e.g., sugars and lipids, which cannot be specifically applied to a synthetic peptide. However, synthetic peptides may be useful for the priming of an immune response, as first demonstrated for poliovirus.[18]

A third novel strategy for the formulation of killed vaccines is the use of anti-idiotypic antibodies (anti-antibodies), whose existence and function in the regulation of the immune response first were articulated by Jerne.[19] Since antibodies bear a structural image of the primary antigen at the antigen-combining site (idiotype), antibodies to antibodies (anti-idiotype) have an antigen-combining site that is structurally similar to the antigen (Fig. 29–6). Thus, inoculation of the anti-idiotypic antibody functions as a vaccine by inducing an anti-anti-idiotypic antibody which in principle should be identical to the first antibody.[20]

This approach has been applied to formulating a vaccine for hepatitis B.[21] While this strategy clearly warrants further study, it suffers from two potential drawbacks. Since the immunogen is an antibody, which is structurally related to naturally occurring human antibodies, problems related to antigenic sensitization must be addressed. In addition, the images borne by anti-idiotypic antibodies are structurally analogous to peptide domains on the surface of the pathogen rather than to whole proteins. Therefore, such

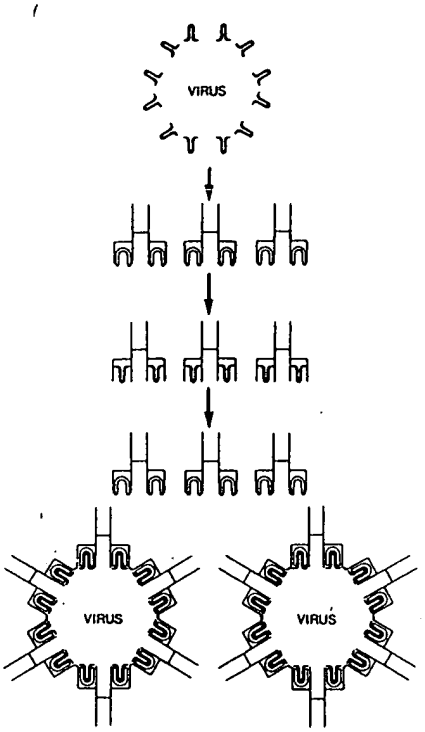


Figure 29–6. Strategy for the use of anti-idiotype antibodies as yaccines.

vaccines might elicit an immune response which is more antipeptide-like in nature rather than antiprotein-like, as discussed previously.

The progression of a vaccine candidate from the laboratory to the marketplace is long and arduous, often taking 10 years from the time of its initial discovery and characterization. Vaccines made by means of new molecular technologies are being developed rapidly. Of these, only rDNA-derived proteins have gone as far as human clinical trials, much less having become a licensed product as in the case of yeast-derived HBsAg. Representatives of such vaccines are listed in Table 29–7, along with others derived by older types of technologies.

With the development of the increasingly so-

Table 29–7. The Progression of Human Vaccines Made by Different Technologies Toward Becoming Licensed Products

	Preclinical Testing	Clinical Testing	Licensed Product	Examples
Live Vaccines				
Classic Strategies				
Modification in cell culture	X	X	X	Measles, mumps, rubella
Variants from other species	X	X	X	Smallpox (vaccinia), rotavirus
Temperature-selected mutants	X	X		Influenza
Reassorted genomes	X	X		Rotavirus
Molecular Strategies				
DNA modification mutants	X			Poliovirus, *Salmonella, Shigella*
Recombinant viruses	X			Vaccinia, herpes simplex, varicella-zoster
Killed Vaccines				
Classic Strategies				
Killed whole pathogens	X	X	X	Pertussis
Toxoids from pathogens	X	X	X	Diphtheria, tetanus, cholera
Purified surface components	X	X	X	Hepatitis B
Conjugated surface components	X	X		Meningitis (*Hemophilus influenzae* b)
Molecular Strategies				
Recombinant-derived proteins	X	X	X	Hepatitis B
Synthetic peptides	X			Hepatitis B
Anti-idiotypic antibodies	X			Hepatitis B, rabies

phisticated analytical tools of molecular biology and immunology, vaccines derived from the newer technologies are receiving closer scrutiny at the regulatory and clinical levels than have vaccines derived from more classic strategies. This trend is expected to continue and represents a formidable barrier for manufacturers to hurdle with respect to the licensing of safe and effective vaccines. As with any technology, there is a learning curve for both manufacturers and regulatory agencies.

A major challenge facing manufacturers and society in the United States is the profound increase in litigation over adverse experiences related to vaccines. The most dramatic manifestation of this litigation is the increased expense and intermittent unavailability of product liability insurance to the three United States–based manufacturers of the vaccine for pertussis, thus resulting in the temporary withdrawal of products of two of these firms from the market and the tripling in the price of the vaccine. Medically, one may find a situation in which the general welfare of the pediatric population may be at significant risk to whooping cough. This problem could have tragic consequences for society and become a severe disincentive for the development of vaccines by means of new technologies. It is hoped that, while legislative remedies to this severe problem are being addressed, research scientists and medical researchers will continue to receive as much support as possible in the pursuit of new technologies, since the vaccines that result represent the most cost-effective products for the eradication of infectious diseases.

REFERENCES

1. Kit S, Kit M, Pirtle EC. Attenuated properties of thymidine kinase-negative deletion mutants of pseudorabies virus. Am. J. Vet. Res. 46:1359–1367, 1985.
2. Omata T, Kohara M, Kuge S, Komatsu T, et al. Genetic analysis of the attenuation phenotype of poliovirus type 1. J. Virol. 58:348–358, 1986.
3. Panicali D, Paoletti E. Construction of poxviruses as cloning vectors: Insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. Proc. Natl. Acad. Sci. (USA) 79:4927–4931, 1982.
4. Mackett M, Smith GL, Moss B. Vaccinia virus: A selectable eukaryotic cloning and expression vector. Proc. Natl. Acad. Sci. (USA) 79:7415–7419, 1982.
5. Roizman B, Jenkins FJ. Genetic engineering of novel genomes of large DNA viruses. Science 229:1208–1214, 1985.
6. Lowe RS, Keller PM, Keech BJ, Davison AJ, et al. Varicella-zoster virus as a live vector for the expression of foreign genes. Proc. Natl. Acad. Sci. (USA) 84:3896–3900, 1987.
7. Buller RML, Smith GL, Cremer K, Notkins AL, Moss B. Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase–negative phenotype. Nature 317:813–815, 1985.
8. Perkus ME, Piccini A, Lipinskas BR, Paoletti E. Recombinant vaccinia virus: Immunization against multiple pathogens. Science 229:981–984, 1985.
9. Eppstein DA, Marsh YV, Schreiber AB, Newman SR, et al. Epidermal growth factor receptor occupancy inhibits vaccinia virus infection. Nature 318:663–665, 1985.
10. Scolnick EM, McLean AA, West DJ, McAleer WJ, et al. Clinical evaluation in healthy adults of a hepatitis B vaccine made by recombinant DNA. J.A.M.A. 251:2812–2814, 1984.
11. Young JF, Hockmeyer WT, Gross M, Ballou WR, et al. Expression of *Plasmodium falciparum* circumsporozoite proteins in *Escherichia coli* for potential use in a human malaria vaccine. Science 228:958–962, 1985.
12. Zavala F, Tam JP, Hollingdale MR, Cochrane AH, et al. Rationale for development of a synthetic vaccine against *Plasmodium falciparum* malaria. Science 228:1436–1440, 1985.
13. Young RA, Mehra V, Sweetser D, Buchanan T, et al. Genes for the major protein antigens of the leprosy parasite *Mycobacterium leprae*. Nature 316:450–452, 1985.
14. Morein B, Helenius A, Simons K, Pettersson R, et al. Effective subunit vaccines against an enveloped animal virus. Nature 276:715–719, 1978.
15. Sutcliffe JG, Shinnick TM, Green N, Lerner RA. Antibodies that react with predetermined sites on proteins. Science 219:660–664, 1983.
16. Merrifield B. Solid phase synthesis. Science 232:341–346, 1986.
17. Lerner RA, Green N, Alexander H, Liu FT, et al. Chemically synthesized peptides predicted from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles. Proc. Natl. Acad. Sci. (USA) 78:3403–3407, 1981.
18. Emini EA, Jameson BA, Wimmer E. Priming for and induction of anti-poliovirus neutralizing antibodies by synthetic peptides. Nature 304:699–703, 1983.
19. Jerne NK. The generative grammar of the immune system. Science 229:1057–1062, 1985.
20. Dressman GR, Kennedy RC. Anti-idiotypic antibodies—Implications of internal image based vaccines for infectious diseases. Inf. Dis. 151:761–775, 1985.
21. Kennedy RC, Eichberg JW, Lanford RE, Dressman GR. Anti-idiotypic antibody vaccine for type B viral hepatitis in chimpanzees. Science 232:220–223, 1986.

PLOTKIN & MORTIMER

VACCINES

VACCINES

Stanley A. Plotkin, M.D.

Chair, Division of Infectious Diseases
The Children's Hospital of Philadelphia
Professor of Pediatrics and Microbiology
University of Pennsylvania
Professor, The Wistar Institute
Philadelphia, Pennsylvania

Edward A. Mortimer, Jr., M.D.

Elisabeth Severance Prentiss Professor of
Epidemiology and Biostatistics
Chairman, Department of Epidemiology and Biostatistics
Professor of Pediatrics
School of Medicine
Case Western Reserve University
Cleveland, Ohio

with 40 contributors

W. B. SAUNDERS COMPANY 1988
Harcourt Brace Jovanovich, Inc.

Philadelphia ◆ London ◆ Toronto ◆ Montreal ◆ Sydney ◆ Tokyo

When Does Homology Mean Something Else?

Molecular biologists routinely compare sequences between different genes, searching for degrees of "homology" between the two; "foul" cry evolutionary biologists, who say the word is misused, thereby causing confusion

"WHEN I use a word," said Humpty Dumpty in a rather scornful tone, "it means just what I choose it to mean—neither more nor less." But, in the scientific realm at least, such an Alice Through the Looking Glass approach to language can lead to misunderstandings: a prime example is the word homology.

" 'Homology' has the precise meaning in biology of 'having a common evolutionary origin,' but it also carries the loose meaning of 'possessing similarity or being matched.' Its rampant use in the loose sense is clogging the literature on protein and nucleic acid sequence comparisons with muddy writing and, in some cases, muddy thinking."

This verbal volley, launched in the pages of *Cell*, is the most recent attack on an old problem. "Yes, this battle has been fought for more than a decade, but it has usually been fought by individuals," says Gerald Reeck of the Kansas State University. "I thought it was time for a more concerted effort." That effort is in the form of an appeal signed by Reeck and ten prominent evolutionarily-oriented molecular biologists, including, Richard Dickerson, Thomas Dukes, Emanuel Margoliash, and Emile Zuckerkandl. "With a collective effort to mend our ways, proper usage [of homology] would soon become fashionable and therefore easy." they write. "We believe that we and our scientific heirs would benefit significantly."

The problem arises in the comparison of sequences, either of proteins or genes, in which, say, a 20% identity of sequence is typically described as 20% homology. "Molecular biologists know what they mean by such a statement," says Walter Fitch, a molecular biologist at the University of Southern California and a cosigner of the letter. "But in fact they are mixing together two different, but related, properties. To classical biologists, homology means not just similarity of structure, it means common descent. It may be true in many cases that similarity of sequences between, say, two genes is the result of common descent, of homology. But I believe it is important to distinguish the observation from the conclusion."

Russell Doolittle, a molecular biologist at the University of California at San Diego, and a cosigner of the letter, traces the abuse of homology back through almost two decades. "Most people in protein chemistry in the late 1960s were not classically trained biologists," he explains, "and to them the word homology simply meant similarity.

"When I use a word," said Humpty Dumpty in a rather scornful tone, "it means just what I choose it to mean –neither more nor less."

This word mutation became fixed in this group, and continues through to today. A second group led by Walter Fitch and others—people with a much greater awareness of evolutionary biology—saw the need for correct usage, and invented all kinds of other terms that simply made the whole thing very complicated and esoteric."

In recent years the sequencing fraternity has far outweighed Fitch and his ilk, and homology meaning similarity has become common usage, as any quick glimpse of the literature reveals. "We've been swamped," says Reeck. At the same time, many molecular biologists have become interested in evolutionary questions. As the Whitehead Institute's David Baltimore once remarked, "everything we look at is evolution." Once this happened, the potential for confusion became acute.

The first skirmish over the use of homology was between Fitch and others in the pages of *Science* some 15 years ago. Fitful exchanges continued in various vehicles, with a flurry of letters breaking out in *Nature* 4 years ago. Reeck's current multi-authored appeal is an attempt to raise the issue beyond the level of individual sparring.

"I've been spending summers collaborating with Chrisoph de Haen and David Teller," says Reeck, "and we would discuss this from time to time. Eventually we decided that a group effort was the only way to achieve anything. The result was the letter to *Cell*. De Haen and Teller, both at the University of Washington, became cosigners.

"People tell us that things have gone too far," says Fitch, "even people who were sympathetic with what we are trying to do. Maybe that's true. I'm not interested in fighting for lost causes. I just think things should be clear."

The clarity Fitch seeks is this. "In its precise biological meaning, 'homology' is a concept of quality. The word asserts a type of relationship between two or more things," write Reeck and his colleagues. That relationship is common descent, and therefore homology cannot be partial—10%, 20%, and so on. "Things can't be partially homologous any more than a woman can be partially pregnant," quips Fitch.

"If using 'homology' loosely did not interfere with our thinking about evolutionary relationships," write Reeck and his colleagues, "the way in which we use the term would be a rather unimportant semantic issue. The fact is, however, that loose usage in sequence comparison papers often makes it difficult to know the author's intent and can lead to confusion for the reader (and even for the author)."

One key source of confusion is that a degree of structural similarity is an irrefutable, quantified fact, supposing the sequencing has been done correctly. By contrast, the suggestion of common descent must always be an hypothesis, however strongly supported by the evidence. Structural similarity and homology are clearly very closely tied together, but they are not necessarily the same thing.

Keeping the two things apart requires using different words, urge Reeck and his colleagues. "Sequence similarity" should be used to describe what is observed between two structures. "Homology" is the inference of common evolutionary origin. Period. The cosigners say that many people argue that attempting to enforce such terminology is anachronistic, that the word homology itself is evolving and taking on new meanings. "If that evolution is toward vagueness and if it results in making our scientific discourse unclear, surely we should intervene." ■

ROGER LEWIN

ADDITIONAL READING

G. R. Reeck *et al.*, "Homology in proteins and nucleic acids: A terminology muddle and a way out of it," *Cell* 50, 667 (1987).

SCIENCE, VOL. 237

Science 237: 1570 (1987)

Cell, Vol. 50, 667, August 28, 1987, Copyright © 1987 by Cell Press

Letter to the Editor

"Homology" in Proteins and Nucleic Acids: A Terminology Muddle and a Way out of It

"Homology" has the precise meaning in biology of "having a common evolutionary origin," but it also carries the loose meaning of "possessing similarity or being matched." Its rampant use in the loose sense is clogging the literature on protein and nucleic acid sequence comparisons with muddy writing and, in some cases, muddy thinking.

In its precise biological meaning, "homology" is a concept of quality. The word asserts a type of relationship between two or more things. Thus, amino acid or nucleotide sequences are either homologous or they are not. They cannot exhibit a particular "level of homology" or "percent homology." Instead, two sequences possess a certain level of *similarity*. Similarity is thus a quantitative property. Homologous proteins or nucleic acid segments can range from highly similar to not recognizably similar (where similarity has disappeared through divergent evolution).

If using "homology" loosely did not interfere with our thinking about evolutionary relationships, the way in which we use the term would be a rather unimportant semantic issue. The fact is, however, that loose usage in sequence comparison papers often makes it difficult to know the author's intent and can lead to confusion for the reader (and even for the author).

There are three common situations in which hazards arise by using "homology" to mean similarity. The first case is the most obvious offense but perhaps the least troublesome. Here an author identifies sequence similarities (calling them homologies) but claims that the sequences being compared are not evolutionarily related. Some awkward moments occur in such a paper, since the author claims both homology (i.e., similarity) and nonhomology (i.e., lack of a common ancestor). Nonetheless, the author's ideas are likely to be clear since arguments against common ancestry are presented explicitly.

A second case is one in which an author points out similarities (again called homologies) but does not address the issue of evolutionary origins. The reader, seeing the term "homology," may infer that the author is postulating coancestry when that is not the author's intent.

The final case occurs most frequently and is the most subtle and therefore most troublesome. Here, similarities (called homologies) are used to support a hypothesis of evolutionary homology. In this case, the two meanings of homology seem to overlap, and it is almost inevitable that the thinking of author and reader alike will be intrusively distorted as follows. Similarity is relatively straightforward to document. In comparing sequences, a similarity can take the form of a numerical score (% amino acid or nucleotide positional identity, in the simplest approach) or of a probability associated with such a score. In comparisons of three-dimensional structures, a typical numerical description is root-mean-square positional deviation between compared atomic positions. A similarity, then, can become a fully documented, simple fact. On the other hand, a common evolutionary origin must usually remain a hypothesis, supported by a set of arguments that might include sequence or three-dimensional similarity. Not all similarity connotes homology but that can be easily overlooked if similarities are called homologies. Thus, in this third case, we can deceive ourselves into thinking we have proved something substantial (evolutionary homology) when, in actuality, we have merely established a simple fact (a similarity, mislabeled as homology). Homology among similar structures is a hypothesis that may be correct or mistaken, but a similarity itself is a fact, however it is interpreted.

We believe that the concepts of evolutionary homology and sequence or three-dimensional similarity can be kept distinct only if they are referred to with different words. We therefore offer the following recommendations:

- Sequence similarities (or other types of similarity) should simply be called similarities. They should be documented by appropriate statistical analysis. In writing about sequence similarities the following sorts of terms might be used: a level or degree of similarity; an alignment with optimized similarity; the % positional identity in an alignment; the probability associated with an alignment.
- Homology should mean "possessing a common evolutionary origin" and in the vast majority of reports should have no other meaning. Evidence for evolutionary homology should be explicitly laid out, making it clear that the proposed relationship is based on the level of observed similarity, the statistical significance of the similarity, and possibly other lines of reasoning.

One could argue that the meaning of the term "homology" is itself evolving. But if that evolution is toward vagueness and if it results in making our scientific discourse unclear, surely we should intervene. With a collective decision to mend our ways, proper usage would soon become fashionable and therefore easy. We believe that we and our scientific heirs would benefit significantly.

Gerald R. Reeck,[1] Christoph de Haën,[2] David C. Teller,[3] Russell F. Doolittle,[4] Walter M. Fitch,[5] Richard E. Dickerson,[6] Pierre Chambon,[7] Andrew D. McLachlan,[8] Emanuel Margoliash,[9] Thomas H. Jukes,[10] and Emile Zuckerkandl[11]

[1]Department of Biochemistry, Kansas State University, Manhattan, Kansas 66506; [2]Departments of Medicine and Biochemistry, University of Washington, Seattle, Washington 98195; [3]Department of Biochemistry, University of Washington, Seattle, Washington 98195; [4]Department of Chemistry, University of California at San Diego, La Jolla, California 92093; [5]Department of Molecular Biology, University of Southern California, Los Angeles, California 90089; [6]Molecular Biology Institute, University of California, Los Angeles, California 90024; [7]Institute of Biological Chemistry, Strasbourg, France; [8]MRC Laboratory of Molecular Biology, Cambridge, England; [9]Department of Biochemistry and Molecular Biology, Northwestern University, Evanston, Illinois 60201; [10]Department of Biophysics, University of California, Berkeley, California 94720; [11]Linus Pauling Institute, Palo Alto, California 94306

06115978 EMBASE No: 1995146710

Advances in vector systems for gene therapy

Hodgson C.P.

Creighton Cancer Center, Department of Biomedical Sciences, Creighton School of Medicine,Omaha, NE 68178 United States

Expert Opinion on Therapeutic Patents (EXPERT OPIN. THER. PAT (United Kingdom) 1995, 5/5 (459-468)
CODEN: EOTPE ISSN: 1354-3776
DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Innovation in gene therapy has become an area of intense interest to biomedical researchers, due to the failure of conventional methods to provide safe, prolonged expression of genes in vivo. Transfection methods provide safe, efficient delivery, but only transient expression. Although viral transduction methods provide permanent insertion of genes into the genome (via retroviruses), they are subject to safety problems, complement inactivation, size limitations and transcriptional inactivation of the viral promoter. This void between the two methods is being fulfilled by new materials and procedures aimed at utilising the best of both transduction and transfection manoeuvres. Synthetic vectors and delivery systems are also being devised to permit more selective targeting on three levels: transductional targeting (entry into desired cells); transcriptional targeting (selective expression); and positional targeting (entry into precise genomic loci). The tools for these advanced molecular conjugate vectors include combinations of polycations, peptide signals, liposomes, viral enzymes, and the appropriate nucleic acid substrates.

DRUG DESCRIPTORS:
*dna
liposome--pharmaceutics--pr; polycation--pharmaceutics--pr; virus enzyme
MEDICAL DESCRIPTORS:
*gene therapy; *cloning vector
genetic engineering; genetic transcription; genetic transduction; medical research; methodology; nonhuman; patent; review; genetic transfection; virus; pharmaceutics; drug delivery system
CAS REGISTRY NO.: 9007-49-2 (dna)
SECTION HEADINGS:
022 Human Genetics

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut
My NCBI (Cubby)

Related Resources
Order Documents
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

1: Trends Genet. 1994 May;10(5):174-8. Related Articles, Links

Gene therapy for cancer.

Culver KW, Blaese RM.

Human Gene Therapy Research Institute, Iowa Methodist Medical Center, Des Moines 50309.

Initiation of clinical trials of gene therapies for cancer has been made possible by two major technological advances: the ability to clone genes that constitute the genetic basis of carcinogenesis or that have therapeutic potential, and the development of an increasing number of gene transfer methods. As a result, 30 experimental trials of gene therapy for the treatment of human cancer have been approved in the United States of America. Here, we discuss the current status of gene therapy for cancer together with future directions for its development.

Publication Types:
- Review
- Review, Tutorial

PMID: 8036720 [PubMed - indexed for MEDLINE]

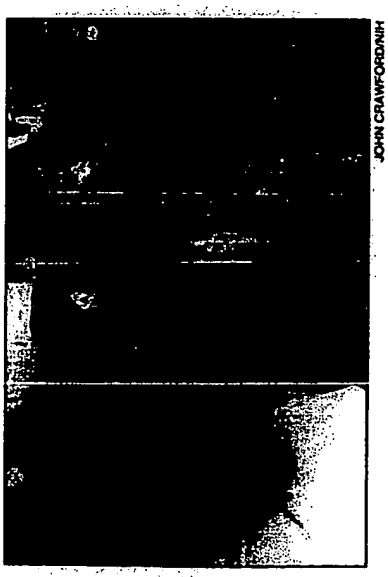
Gene Therapy's Growing Pains

With more than 100 clinical trials started and hundreds of millions of dollars at stake, the field is struggling to meet expectations

Last September, when Ashanthi DeSilva, a cheerful 8-year-old, appeared before the House Science Committee, the panel's chair at the time, Representative George Brown Jr. (D–CA), was moved to declare that she was "living proof that a miracle has occurred." DeSilva made history in 1990 when she received the first authorized human gene therapy ever attempted. She had been born with a defective version of the gene that normally makes the essential enzyme adenosine deaminase (ADA)—a condition that, if left untreated, causes a fatal malfunction of the immune system. Four years after receiving her first injection of cells containing functioning ADA genes, Ashanthi, apparently in good health, was chatting with members of Congress.

Since that epic treatment, gene therapy has taken off like a rocket. More than 100 clinical trials, aimed at treating conditions ranging from inherited disorders such as cystic fibrosis to cancer and AIDS, have been given the go-ahead. The National Institutes of Health (NIH) is spending an estimated $200 million a year to develop and test tools and techniques for gene therapy. Private companies have raised hundreds of millions of dollars to enter the field and are now sponsoring most of the clinical trials. Many academic centers have created gene-therapy programs and joined the jockeying for a piece of the action.

Yet in spite of this enthusiasm—bolstered by media hype—all is not well in the world of gene therapy. So far, there has been no unambiguous evidence that genetic treatment has produced therapeutic benefits. Even data from the pioneering ADA trials are not decisive: Ashanthi and the other children who have since been treated with gene therapy are also being given routine injections of synthetic ADA, and these conventional treatments may be responsible for their good health (see box on p. 1051). Gene therapists are still encountering difficulties in transferring genes to adequate numbers of target cells and getting them expressed. This problem afflicts all areas of gene therapy, but it has become acute in efforts to treat cystic fibrosis (CF): Several CF protocols have been revised because of side effects that may have been triggered by the adenovirus agent used to transfer genes, and some researchers say that adenovirus-based therapy for CF must now be rethought (see box on p. 1052).


JOHN CRAWFORD/NIH

High hopes. The first attempt, in 1990, to correct an ADA gene defect.

Faced with such fundamental problems, several biomedical leaders, including NIH Director Harold Varmus, are saying it's time for NIH to pause, examine what gene therapy has accomplished, and determine what role NIH should be playing in the field. "Despite the growing support for gene therapy," Varmus said at a public meeting in May, the field "remains at a very early stage of development. While there are several reports of convincing gene transfer and expression, there is still little or no evidence of therapeutic benefit in patients—or even in animal models." Nor, he added, is there a consensus about which gene delivery systems will be most effective, and he said he wasn't confident the field was choosing the best lines of attack.

Of particular concern to Varmus and some leaders in the field is the possibility that the intense commercial interest in gene therapy is prompting a stampede into clinical trials and pressure for quick results—before the basic science has been worked out. Drew Pardoll, a Johns Hopkins University co-investigator in a gene-therapy trial for prostate cancer, deplores the lack of rigor in many studies. "There's been an emphasis on glitz," he says. "It's produced a culture in which getting into clinical trials—getting into the club—has been more important than getting a meaningful result."

These concerns have prompted the most intensive review of this burgeoning field since that first ADA experiment 5 years ago. Earlier this year, Varmus created two high-level panels to advise him on how NIH should proceed. The first, chaired by Inder Verma, a geneticist at the Salk Institute, is looking into NIH's procedures for approving gene-therapy clinical trials (see box on p. 1054). The second, co-chaired by Arno Motulsky, a geneticist at the University of Washington, Seattle, and Stuart Orkin, a hematologist at Harvard University, has been asked to chart a strategy for how NIH should invest in gene therapy, choose areas to emphasize, and help shape guidelines for medical practice. Both panels will issue recommendations by December.

The Motulsky-Orkin panel is drawing a lot of interest—and some nervousness—from gene-therapy researchers in part because Varmus deliberately set it up to take an independent look at the field. Varmus chose its members, he said, for their "stature in the scientific community" and because none is directly involved in running a gene-therapy company or clinical trial.

Varmus's intramural adviser on gene therapy, virologist Nelson Wivel, director of the NIH Office of Recombinant DNA Activities, says he "would not be surprised" if the panel suggests backing off from the heavy emphasis on clinical trials today. Instead, Wivel suggests, the panel may stress the importance of funding basic virology and immunology. "This is the primary question," Wivel says: "Should you be doing more [clinical] trials before you've solved other major technical issues," such as making vectors more efficient and less toxic? These recent developments at NIH, the cradle of gene therapy, suggest the soaring enthusiasm for clinical experimentation may be cooling.

A glass half full?

That enthusiasm is still very visible these days—particularly in the media. "Gene Therapy Techniques Advance as Potential Treatments for Cancer," reported *Genetic Engineering News* on 1 March. "The Birth of a Megamarket," proclaimed *Fortune* on 15 May, featuring Canji Inc., a gene-therapy company in San Diego. "Gene Therapy May One Day Help Doctors Fix Ailing Hearts," announced Johns Hopkins University on 28 July. "Gene Therapy Boosts Radiation Therapy for Cancer," said a University of Chicago press release on 31 July.

Beginning with a wave of media attention

Jury Still Out on Pioneering Treatment

Every time physician Melissa Elder opens a vial of the enzyme she injects into two young brothers she treats, it costs $2200. Elder says the two boys use a total of five vials a week; it costs more than $40,000 a month to keep them healthy.

These brothers—Rhett, age 4, and Zach, age 2—lack a gene that expresses the enzyme adenosine deaminase (ADA), essential to the immune system. Failure to produce ADA leads to a deadly condition: severe combined immunodeficiency disease. To fend it off and keep infection at bay, Elder, an immunologist at the University of California, San Francisco, treats Rhett and Zach with a synthetic form of the enzyme known as PEG-ADA.* She says the parents are acutely aware of their sons' vulnerability—and of the cost of using PEG-ADA: "The parents lose sleep worrying about what will happen when their insurance reaches its cap." The policy has a limit of $1 million, already half spent.

This is exactly the kind of misfortune gene therapy is meant to prevent. But it hasn't in this case: Zach has received gene therapy to replace missing ADA genes since shortly after he was born. Like the other children who have been given ADA gene therapy in the United States and overseas, he still gets weekly injections of PEG-ADA. Even the two girls who made history 5 years ago as the first patients to receive ADA gene therapy receive PEG-ADA shots. The reason: Physicians have seen other children's immune function decline when PEG-ADA was reduced, and they worry that it would risk the children's health to rely on gene therapy alone.

Elder and other physicians treating the handful of children who have been given gene therapy for ADA deficiency say their patients' health has improved. But as long as the children continue to get PEG-ADA shots, researchers cannot say for sure how much of the credit should go to the gene therapy.

Proof seeker. NIH's Blaese aims to prove ADA therapy works.

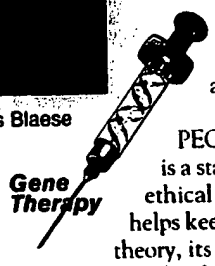
Even principal investigators in the gene therapy trials—Michael Blaese of the National Institutes of Health (NIH) and Donald Kohn of the Los Angeles Children's Hospital—agree that the mixed treatment clouds the role of gene therapy. "There are a lot of questions to be answered," Blaese concedes. But he argues that, in the case of his first two gene-therapy patients, "the experiment was valuable irrespective of whether [the children] were on enzyme or not." He says the experiment proved that it's possible to transfer corrective genes to humans and to get the genes to express ADA "at a very good level" in at least one patient—Ashanthi DeSilva—for several years.

Ashanthi was given her first dose of gene therapy in 1990; a second patient was treated in 1991. Both were also put on PEG-ADA, approved as a standard therapy in 1990 by the Food and Drug Administration. In attempting gene therapy, Blaese and a team at NIH focused at first on T cells circulating in the girls' bloodstream—removing blood, treating T cells with stimulants, inserting a new ADA gene, and infusing the cells back into the patients. Each girl received 11 to 12 treatments. Blood tests conducted 3 years later showed that more than 50% of Ashanthi's circulating T cells contained the new gene, says Blaese.

But, in a telling indication of the hit-or-miss nature of this new technology, only 0.1% to 1% of the other patient's did. Clinical signs have improved in both girls, however. In Ashanthi's case, "it's very hard to say this was due just to enzyme [PEG-ADA]," says Blaese, although he recognizes that in the other case, "there just isn't enough" of the new gene present for it to deserve much credit.

Other researchers say it's easy to overestimate gene therapy's contribution. Ricardo Sorensen, a physician at the Louisiana State University Medical Center who treats ADA-deficient children, notes that the infusion of stimulated T cells alone may have been beneficial for these young patients and that the PEG-ADA must have helped. The best way to sort out what each treatment did, says Sorensen, would be to give T cell therapy, PEG-ADA therapy, and gene therapy independently to patients with similar conditions. Short of that, says Michael Hershfield, the Duke University researcher who developed PEG-ADA, a good way to get an answer would be to withdraw PEG-ADA from children who have received gene therapy and see how they do. That is exactly what Blaese and his colleague Kohn are doing right now.

Together, they have been running an experiment in which Zach and two other ADA-deficient boys were given a new type of ADA gene therapy in 1993, at their birth. In all three cases, researchers removed blood from the children's umbilical cord and attempted to inject an ADA gene into long-lived "stem" cells, which give birth to other blood cells and are relatively abundant in cord blood. The goal: to create a permanent source of ADA-competent T cells. Preliminary data suggest partial success: Up to 10% of their circulating T cells now seem to carry a healthy gene, and Kohn says the hope is that, with time, these healthy cells will accumulate and predominate.



These children have also been receiving PEG-ADA since birth, says Kohn, because "it is a standard therapy, and we felt it wouldn't be ethical to withhold it." However, the PEG-ADA helps keep genetically defective cells alive, and in theory, its use retards the rate at which they can be cleared from the bloodstream to make room for healthy cells. For this reason, Blaese and Kohn are eager to see the boys' PEG-ADA shots curtailed. Since January, Blaese says, the level of PEG-ADA given these three patients has been cut in half (to 30 units per kilogram per week). By the end of the year, he had hoped to cut it close to zero.

But the experiment is not advancing as rapidly as Blaese would like. Physicians for all three boys—including Elder—say they are reluctant to cut the PEG-ADA doses below the present level. Elder, for example, says: "The more PEG-ADA I give, the better the white cell count" and the stronger the immune function. Already the patients' white cell counts have dropped with the initial decline in PEG-ADA doses, although the fraction of "cured" T cells has increased. Physicians are watching closely to see whether the boys can tolerate further reductions before allowing the experiment to proceed. If so, and if the transplanted genes eventually provide all the ADA Zach and the other two boys in this test require, it would be the first unambiguous demonstration that gene therapy has cured a patient's disease.

–E.M.

* Polyethylene glycol–ADA, bovine ADA with artificial surfaces added to prolong life in the bloodstream, manufactured as Adagen by Enzon Inc. of Piscataway, N.J.

generated by NIH's attempt to fix Ashanthi DeSilva's defective ADA gene 5 years ago, encouraging reports like these have swelled to a flood. Most such reports are based on research developments that have yet to be tested in clinical trials, however. And the clinical trials that have been conducted over the past 5 years have yielded very few published results—so few that the Motulsky-Orkin panel will have little hard data to analyze as it tries to figure out how the field is progressing.

NIH's Recombinant DNA Advisory Committee (RAC), which reviews all NIH-funded clinical research protocols for gene therapy, discovered for itself the paucity of data when it established a subcommittee to see where the field is heading. The panel, led by Brian Smith, a Yale University oncologist and RAC member, and NIH staffer Debra Wilson, scanned all trials approved by the RAC and the Food and Drug Administration (FDA) through June 1995. The panel found little concrete information on the results of these trials, but it did paint a remarkable picture of how rapidly the field has grown—both in terms of the numbers of trials and the wide range of disorders gene therapists are boldly trying to treat.

The RAC team found that 567 patients are involved in 106 RAC-approved experiments. Almost half (268) are new recruits, having entered trials since December 1994. Only a small fraction of these experiments are aimed at correcting defective genes. Instead, most protocols aim to induce specific cells, such as cancer cells or cells infected by HIV, to produce proteins that would make them vulnerable to attack by the immune system. Others are attempting to use gene therapy as an adjunct to traditional chemotherapy for cancer (see chart on next page).

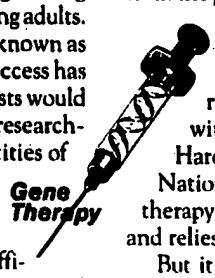
The field, in short, has moved a long way from the popular notion of gene therapy as a cure for genetic disease. Indeed, the RAC panel identified only 20 trials focusing on single-gene deficiencies such as ADA. Of these, 11 aim to replace the defective chloride transport gene that causes cystic fibrosis, using an adenovirus vector to shuttle functioning genes into a patient's lung cells. Three other trials aim to treat Gaucher disease, a metabolic disorder; single trials are aimed at each of six other rare diseases including ADA deficiency. Little has been published from these efforts: Only preliminary data have seen the light of day in peer-reviewed journals.

In contrast to the few efforts aimed at single-gene disorders, almost half the 106 trials are aimed at cancer. One reason for the

The Trouble With Vectors

Cystic fibrosis (CF) is a lethal inherited disease for which gene therapy offers a rare hope of relief. CF patients—of whom there are more than 30,000 in the United States—lack a gene that enables cells to process the chloride ion, causing their lungs to be plagued by mucus and infection. Gene therapy's promise is that one day it may be possible to replace defective genes with healthy ones, lengthening the lives of CF patients, who generally die as children or young adults.

Already, researchers have transferred a working gene (known as *CFTR*) into the surface airway cells of lab animals. This success has inspired 11 human trials. But any expectation that these tests would quickly demonstrate therapeutic benefits has dwindled as researchers have run into problems in transferring sufficient quantities of the *CFTR* gene into patients' cells. In addition, the virus vector they are using as the transfer agent has provoked an immune reaction in some patients.



CF researchers are not alone in encountering such difficulties. Indeed, right from the start, gene therapists have recognized that their central challenge would be to find safe vectors capable of transporting genes efficiently into target cells—and getting the cells to express the genes once they are inserted. Although there have been promising developments in some areas, it remains the central challenge for every area of gene therapy. Francis Collins, director of the National Center for Human Genome Research (NCHGR), sums up the situation bluntly: "None of the currently available techniques is clinically useful for systemic gene delivery," the kind that can provide a permanent cure. For that reason, NCHGR has joined in a major program to improve vectors and make them available to clinicians (*Science*, 11 August, p. 751).

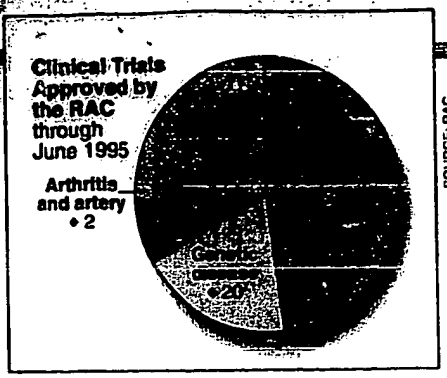
The most popular vector used so far is one based on a retrovirus that normally infects mice. A crippled version of this retrovirus, loaded with therapeutic genes, has been used in 76 of the 106 human trials approved to date, most of which involve patients with cancer or HIV. This mouse virus is the most efficient agent yet identified for transferring genes, although rates of transfer and expression vary dramatically in different patients (see p. 1051).

The stark variation among patients isn't the only problem. Another is that retroviruses insert genes only into cells that are actively dividing and growing, such as T cells. This rules out their use for treating diseases such as CF, where the target cells aren't dividing. A second drawback is that retroviruses insert themselves randomly into host DNA, posing what's thought to be a small—but real—risk of cancer. If a retrovirus gene should settle alongside an oncogene or tumor suppressor gene, it might trigger tumor formation by turning on or off the native gene. For these reasons, retroviral vectors have been used in "ex vivo" procedures—in which cells are removed from the patient, treated, and replaced—and when increased risk of cancer is not considered an obstacle to therapy.

In contrast to those trials, therapy for CF patients has relied primarily on a vector based on a crippled adenovirus. This DNA virus infects 75% of young people, usually without causing illness, according to adenovirus expert Harold Ginsberg, emeritus of Columbia University, now at the National Institutes of Health (NIH). It's attractive for CF therapy because it seeks the lungs. It penetrates nondividing cells and relies on these host cells to express viral DNA.

But it, too, has drawbacks. Adenovirus genes express proteins that trigger immune responses. In consequence, large concentrations of wild virus—and even crippled virus—provoke inflammation along with an immune attack that neutralizes cells containing adenovirus genes. For this reason, the effects of adenovirus vector therapy are likely to be short-lived, lasting about 6 weeks. And, because the immune system "remembers" antigens and attacks them with extra vigor on a second encounter, repeat dosing with adenovirus vector seems impractical at present—unless a strong immune response is desired, as in some types of cancer therapy.

Research on CF gene therapy by Richard Boucher and colleagues at the University of North Carolina, Chapel Hill, indicates that clinicians using adenovirus as a vector are caught between two problems. When administered at low concentrations it is inefficient: The virus doesn't get into many human nasal cavity or airway cells, and few cells express the corrected *CFTR* gene. At high doses, however, it appears to cause acute inflammation, Boucher says. He notes that three or four CF gene therapy trials have been compelled to stop or adjust doses to deal with acute reactions in patients. Some researchers, such Ronald Crystal, who pioneered this field at NIH and is now at the Cornell University Medical Center in New York, think past problems with CF therapy may not involve fundamental issues so much as a need to find the right way to deliver existing materials. But Boucher and Ginsberg believe immunogenicity has been and continues to be a fundamental problem.



SOURCE: RAC

Broad focus. The majority of trials now target diseases with large patient populations.

growing emphasis on this disease, says Wivel, is that private investment in gene therapy is increasing, and companies can't justify large R&D expenses unless they can expect to treat large patient populations. Another reason is that these patients often have no alternatives in conventional medicine and are therefore eligible for experimental therapy.

Thirty of the 51 cancer trials are designed to insert into tumor cells a gene expressing a substance such as the lymphokine interleukin-2 (an immune-system signaling molecule), in the expectation that it will stimulate a natural immune attack on the tumor cells. Another 11 studies aim to induce tumor cells to express the herpesvirus protein thymidine kinase, which makes them vulnerable to treatment with the drug gancyclovir. The remaining 10 trials test three other strategies, including four trials that seek to stop cancer by activating tumor suppressor genes. No results have yet been published from these trials.

Another fast-growing area is gene therapy for AIDS. Indeed, the majority of patients enrolled in clinical trials approved in the first half of 1995—168 out of a total of 268—are participating in tests of an anti-HIV therapy sponsored by Viagene Inc. of San Diego. (These include a trial approved only by FDA; private trials need not obtain RAC approval.)

Viagene has focused on a succession of strategies in at least four RAC-approved HIV trials. These studies aim to put genes that express HIV proteins into some of a patient's cells, in the hope that the cells will express antigens that will prime the immune system to attack infected cells carrying the same antigens. In addition to Viagene's trials, five others go after HIV with other strategies: They seek to disrupt viral functions by creating decoy molecules to compete with, sequester, or cleave products produced by HIV, or they try to cause HIV-infected cells to express thymidine kinase or other molecules that make them targetable by chemical attack. Clinical results have not yet been published from any of these trials.

These two vector types—retrovirus and adenovirus—account for more than 85% of those used in clinical trials. But leading researchers and a few companies are looking for other vehicles. For example, Joseph Glorioso, director of the gene-therapy program at the University of Pittsburgh, is focusing on herpesvirus. It infects the central nervous system and carries a remarkable "latency gene" that hides it from immune attack. In theory, herpesvirus vector could be used to insert DNA into the nervous system. But it is difficult to manipulate and may have hidden risks. Eventually, Glorioso hopes to seek approval to use it for cancer therapy.

R. Jude Samulski, leader of the gene-therapy program at the University of North Carolina, is investigating adeno-associated virus (AAV). It has no known toxicities and replicates only in the presence of a "helper virus," making it look very safe. It may have other advantages, says Samulski: It is simple, and its "life cycle suggests it may be able to persist and deliver genes for a long time."

But Samulski acknowledges a common criticism: AAV is difficult to produce in large quantities. One leading gene-therapy researcher who asked not to be named claims that current AAV technology is inefficient and expensive, adding: "AAV is like an onion—the more layers you peel off, the more you cry." So far, only one human trial using AAV—for treatment of cystic fibrosis—has been approved; no data are yet available. But Samulski predicts, "You'll see more and more AAV protocols."

Viagene Inc. of San Diego is investigating sindbis—an African virus that infects the nervous system—as a new vector. Its virtues include a unique and highly productive method of replication. Other groups are looking into HIV as a vector, although probably only for treating HIV patients.

The hottest alternatives to viruses are oily substances known as cationic liposomes. These concoctions, which come in many varieties, can slip DNA into the cell's nucleus and cause genes to be expressed. One skilled user of the technology, Gary Nabel, a Howard Hughes Medical Institute investigator at the University of Michigan, predicts liposomes will improve dramatically in the next few years, increasing levels of gene expression by "an order of magnitude or more," and that they will quickly be adapted for clinical use. In addition to liposomes, two other nonviral vectors have been adopted for clinical trials: direct injection of plasmid DNA into the muscle (two trials) and air-gun injection of a DNA-coated pellet (one trial).

These examples should make clear that there is as yet no perfect vector. And many researchers say we shouldn't expect one: Instead, there will be a confusing array of viral bits and pieces, combined with other gene-transfer agents, all of them used in custom tools designed for specific applications. But if a perfect vector were to be created, it might look something like the one being pursued by the intramural research staff at NCHGR.

NCHGR has launched a project, led by Melissa Rosenfeld and Paul Liu, to develop what they call a "human artificial chromosome." The idea is to create a synthetic 25th chromosome, big enough to transport whole "suites" of genes into the nucleus of a target cell, including all the regulatory sequences that surround a critical gene. Collins told a review panel in May that NCHGR is collaborating with a few extramural groups in an "intense" effort to push this "high-risk" project forward. But a staffer notes that it hasn't yet achieved "proof of principle." For the present, Collins observed, "the paucity of clinically acceptable gene transfer techniques severely limits the potential applications of gene therapy."

–E.M.

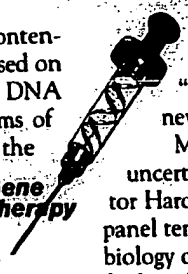
VECTORS IN RAC-APPROVED CLINICAL TRIALS

SOURCE: RAC

Vector	Number of clinical trials	Pluses	Minuses
VIRAL			
Retrovirus	76	Efficient transfer Easy to make	Small capacity Random DNA insertion Dividing cells only Replication risk
Adenovirus	15	Nondividing cells Possibly targetable	Immunogenic Replication risk
Adeno-associated virus	1	Nonimmunogenic	Small capacity Hard to make
Herpesvirus	0	Nonimmunogenic Targets CNS	Risks unclear Hard to make
NONVIRAL			
Liposomes	12	No replication Nonimmunogenic	Low efficiency
[illegible]	3	[illegible]	[illegible]

RAC's Identity Crisis

From the start, gene therapy has been one of the most contentious fields in biomedicine. In the early days, debate focused on safety—on the possibility, for example, that engineered DNA might create novel infectious viruses or trigger new forms of cancer. To minimize such risks and reassure the public, the National Institutes of Health (NIH) beginning in 1980 asked all government-funded gene therapy researchers to submit protocols for prior approval by a public panel. Today, these reviews are carried out by NIH's Recombinant DNA Advisory Committee (RAC), a mixed group of 20 scientists and nonscientists who meet quarterly at NIH. RAC has voted on virtually every gene therapy trial in the United States.



Now, after 5 years of clinical experimentation and no evidence that gene therapy poses a general risk to the public, fears are fading and, with them, the justification for RAC. Some leaders in gene therapy—especially researchers eager to get experiments launched and companies with large sums hanging on clinical trials—are saying it's time for RAC to think about retiring. They point out that the law already requires the Food and Drug Administration (FDA) to monitor clinical trials and clear therapeutic products for safety and efficacy, which means that gene therapy has to pass two federal checkpoints.

Stephen Marcus, an executive at Genetic Therapy Inc. of Gaithersburg, Maryland, says, for example, that RAC delays by 2 months or more decisions ultimately made at the FDA. "It may be a little hyperbolic" to suggest that lives are being lost as a result, Marcus says, "but the concept is there." Likewise, Thomas Reynolds of Targeted Genetics in Seattle complains that RAC has become a "bottleneck." He'd rather see it focus on "novel issues, like germ-line therapy, new vector systems, new disease targets"—not on reviews of individual protocols.

Members of the RAC themselves have also expressed uncertainty about the role they're expected to play, NIH Director Harold Varmus has observed. While public members of the panel tend to focus on safety and ethics, those with expertise in biology often zero in on technical aspects of proposals that they find inadequate. RAC has thus suffered from a split personality. To clarify the committee's proper role, Varmus has taken a couple of steps in the past year. First, he has asked RAC's staff and the FDA to work out a unified review process, now being put into effect, that may allow researchers to submit a single application for review by both agencies. Only those that raise new technical or ethical issues would be debated by the RAC.

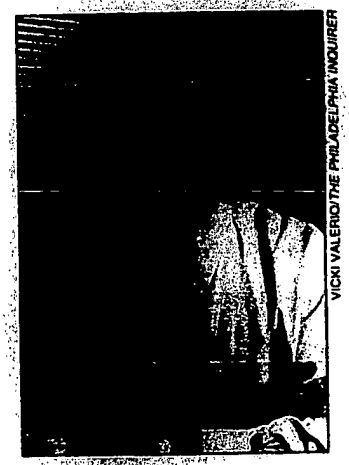
Second, Varmus has appointed a special ad hoc study group—headed by oncogene researcher Inder Verma of the Salk Institute in La Jolla, California—to consider how NIH should review gene therapy in the future. One of the big questions to be addressed is: Who, if anyone, should scrutinize clinical trials for scientific value? Officially, that isn't RAC's job, although expert members find it hard not to comment on technical quality. The Verma panel will deliver its recommendations on this and other broad questions about how NIH should handle gene therapy trials to Varmus by December.

–E.M.

Most of the other trials reviewed by RAC are not aimed at delivering therapy: They are designed to tag specific cells with genetic markers to provide information about the fate of the cells. When RAC members sifted through the catalog of these "gene marking" trials in June, they found that although this area gets little public attention, it is in fact scientifically the most encouraging area. Smith says they have produced at least four peer-reviewed publications laden with "hard data." The research has shown, for example, that cancer relapse following autologous bone marrow transplants—in which a patient's bone marrow is removed before intensive chemotherapy and later replaced—often is caused by tumor cells that survive in the marrow. It indicates that clinical research should zero in on ways to purge tumor cells from the marrow.

Whether this overall picture is judged positive depends in large measure on who's being asked. Pioneer gene therapists and industry leaders tend to view the explosion of trials as evidence of progress. Independent academics, on the other hand, often see the glass as half empty. But both sides can agree that, at the least, the field isn't harming its patients. Clinical trials, says Smith, have shown few signs of toxicity and no hints of runaway genetic mutations: "There are no three-headed cows" of the kind anticipated in "*National Enquirer*–land," he jokes. But the disappointing news, Smith finds, is that so far only hints of therapeutic benefit have appeared.

Wivel notes that nearly all the gene-therapy trials so far have been "phase I" trials, designed to test safety rather than efficacy. So they can't really be judged on effectiveness. But that hasn't discouraged some gene-therapy leaders from trying. Stephen Marcus, a vice president at Genetic Therapy Inc. of Gaithersburg, Maryland, cites a brain cancer patient who, after surgery for glioblastoma, was treated with GTI's anticancer gene therapy and has survived for more than 2 years. This is almost unheard of, Marcus says, and is clearly "a case where there is some evidence of effectiveness." He notes, however, that "we realize this is anecdotal."


VICKI VALERIO/*THE PHILADELPHIA INQUIRER*

Back to basics. James Wilson wants less hype, more research.

But the RAC members who reviewed cancer trials—Robert Erickson of the University of Arizona and R. Jude Samulski of the University of North Carolina—deemed it "too early" to reach any conclusion. Erickson found several unpublished reports that gene therapy had reduced tumor size, but noted that other, simpler therapies have produced similar reports in the past. Samulski pointed to a common theme running through the cancer studies that raised some concern: low rates of gene transfer.

Indeed, difficulties in getting genes transferred efficiently to target cells—and getting them expressed—remain a nagging problem for the entire field. Virus-based vectors have been the most efficient for inserting genes into cells in the lab, but they have run into problems in the clinic. Often the fraction of cells receiving the new gene is low, particularly if these targets of gene therapy are long-lived "stem" cells that give birth to other cells. Researchers say it has been difficult to achieve a 1% rate of gene transfer into stem cells, for reasons not fully understood. And even when genes are inserted in stem cells, they may not be active in second-generation cells, yielding less-than-adequate therapy.

Boosting the rate of gene transfer by increasing the concentration of vector or by dosing patients repeatedly may create another problem, however: It may stimulate the immune system to attack and neutralize the therapy-bearing cells. Francis Collins, director of NIH's National Center for Hu-

man Genome Research, told the Motulsky-Orkin panel in May that "many problems must be solved before gene therapy will be useful for more than the rare application."

Voting with their checkbooks

Academic researchers are still grappling with many fundamental issues in gene therapy. But industry leaders and their financial agents are gung-ho. Investors have poured hundreds of millions of dollars over the past 5 years into gene-therapy companies, drawn by hopes of blockbuster discoveries. And big companies are now getting into the act. Late last year, Switzerland's Ciba-Geigy Ltd. acquired a 49.5% share of Chiron Corp. of Emeryville, California, which then turned around in April 1995 and began buying Viagene. Less than 3 months later, another Swiss pharmaceutical giant, Sandoz AG, bought GTI—an investment that gives Sandoz rights to GTI's broad patent for "ex vivo" therapy, in which cells are removed from the patient, given new genes, and replaced (*Science*, 31 March, p. 1899). Also last fall, the French company Rhône-Poulenc Rorer struck agreements with a network of small companies to gain access to the latest research (*Science*, 18 November 1994, p. 1151).

One result of this burgeoning investment is that private companies have come to dominate clinical trials of experimental gene therapies. By June, according to the RAC, 13 firms had won approval to run at least 34 gene-therapy trials—so that now, 60% of all therapeutic trials are privately funded. Industry also plays an indirect role in physician-sponsored trials, supplying vectors at little or no cost.

U.S. GENE THERAPY TRIALS SPONSORED BY INDUSTRY

Company Sponsor	Founded	Capital ($millions)	No. of Trials	Topic
Applied Immune Sciences	1982	209	1	Cancer
Canji	1990	21	1	Cancer
Cell Genesys	1988	103	1	HIV
GenVec	1992	20	1	Cystic fibrosis
GeneMedicine	1992	50	1	Alpha-1 anti-trypsin
Genetic Therapy	1986	103	6	Cancer, CF, Gaucher
Genzyme	1981	74	5	Cancer, CF, Gaucher
Immune Response	1986	128	1	Cancer
Ingenex	1992	5	2	Cancer
Introgen Therapeutics	1993	NA	3	Cancer
Somatix Therapy	1979	102	3	Cancer
Targeted Genetics	1989	46	1	HIV
Viagene	1987	106	6	Cancer, HIV
Vical	1987	46	4	Cancer, HIV

SOURCE: COMPANY DATA AND RAC

This trend is worrying some leaders in the field, who say biotech companies are forcing the pace and direction of research, and not always in ways anchored in the best science. Varmus, for example, says that while it's "a good thing" that investors are willing to pick up the tab for "very expensive" clinical experiments, these trials absorb "a lot of resources and talent," and he isn't sure that they "are scientifically as worthy as other things that could be done." He's concerned about understanding the biology of viruses used to transfer genes and of the immune reactions they provoke.

Varmus isn't alone in expressing concerns. James Wilson, director of the Institute for Gene Therapy at the University of Pennsylvania, says private funding is important, but he worries that expectations may be raised prematurely. People who invest in gene therapy anticipate a big payoff, but they may not realize how long it will take, Wilson says. "The actual vectors—how we're going to practice our trade—haven't been discovered" yet, he notes, "so it may be early for the impatience of venture capital–supported biotech."

This commercial pressure may also account for some of the hype surrounding developments in gene therapy, says Wilson. If you're the leader of a gene-therapy company, "you try to put as positive a spin as you possibly can" on every step of the research process, he notes, "because you have to create promise out of what you have—that's your value." But, Wilson says, "that's not what we need right now." What the field needs is "a lot of basic research on vectors and cell biology."

Pardoll of Hopkins is equally critical; he says that in the rush to get trials approved, "biological principles are not well thought out—especially immunological principles." Varmus says this happens because the main concern of small companies is to survive, and "one way to survive is to have a clinical trial—show that you're actually on the scoreboard." But promoting the company doesn't necessarily promote gene therapy, Varmus notes: "We're not talking about an industry that's in an advanced state of competence."

Another effect of commercial investment, some researchers say, has been to channel energy into intellectual property disputes and turf battles. For example, Dusty Miller, a virologist at the Fred Hutchinson Cancer Research Center in Seattle, argues that the gene-therapy patent issued to NIH and GTI in April will have a "chilling effect" on research. The patent covers all forms of ex vivo therapy. Miller—who was among those involved in the research that led to this patent but was not named as a co-inventor—calls it "the patent from hell" because it's so broad. He thinks it may discourage newcomers and stifle collaboration. When Miller made this comment at a RAC meeting in June, GTI President James Barrett rose to say the company considers the patent "valid" and will negotiate reasonable terms that are "idiosyncratic" for each use.

Academic scientists may think it's too early to be talking about financial returns, but not company executives and some industry analysts. Take Wall Street biotech analyst Jeffrey Swarz of the investment bank CS First Boston. Swarz delivered an enthusiastic assessment of the field at last year's congressional hearings and was equally bullish in a recent interview with *Science*. Gene therapy for cystic fibrosis, he said, "has been successful; ADA disease has been successful; brain cancer has been successful. ... So far, the technology looks fabulous." He predicts a gene-therapy product will reach the market by next year.

At a recent meeting in Washington, D.C., organized by the Institute for International Research, gene-therapy business chiefs were asked when they thought their industry's first product would hit the market. Few were as optimistic as Swarz, but the forecasts ranged from very soon—in 1997, according to David Nance, president of Introgen Therapeutics of Austin, Texas—to reasonably soon—in 2000, according to Harvey Berger, chair of Ariad Pharmaceuticals in Cambridge, Massachusetts. One attendee, Mark Edwards, managing director of Recombinant Capital, an independent San Francisco firm that analyzes biotech companies, was less ebullient, saying he didn't expect a commercial product until 2003. Whether one's an optimist or not, concluded Berger, "we've got to make sure the biology matches the enthusiasm."

Many academic gene therapists agree with Berger, and some have said they hope the critical review Varmus has ordered from the Motulsky-Orkin panel will cut through the hype that surrounds the field and inform the public that it could be many years before the money invested in clinical trials yields a product. "It may be time for some realism," says Michael Knowles of the University of North Carolina's cystic fibrosis program. Adds Joe Glorioso, director of the University of Pittsburgh's gene-therapy program: "We just can't be wimpy about this; we have to be in for the long haul."

–Eliot Marshall

Targeted vectors for gene therapy

NICHOLAS MILLER[1] AND RICHARD VILE

Laboratory of Cancer Gene Therapy, Imperial Cancer Research Fund, Rayne Institute, St. Thomas' Hospital, London SE1 7EH, United Kingdom

ABSTRACT **Successful gene therapy requires not only the identification of an appropriate therapeutic gene for treatment of the disease, but also a delivery system by which that gene can be delivered to the desired cell type both efficiently and accurately. Reductions in accuracy will inevitably also reduce efficiency since fewer particles will be available for delivery to the correct cells if many are sequestered into nontarget cells. In addition, the therapy will have net benefit to the patient only if gene delivery is sufficiently restricted such that normal cells are left unaffected by any detrimental affects of bystander cell transduction. Here we review how currently available delivery systems, both plasmid and viral, can be manipulated to improve their targeting to specific cell types. Currently, targeting is achieved by engineering of the surface components of viruses and liposomes to achieve discrimination at the level of target cell recognition and/or by incorporating transcriptional elements into plasmid or viral genomes such that the therapeutic gene is expressed only in certain target cell types. In addition, we discuss emerging vectors and suggest how gene therapy delivery systems of the future will be composites of the best features of diverse vectors already in use.— Miller, N., Vile, R. Targeted vectors for gene therapy. *FASEB J.* 9, 190-199 (1995)**

Key Words: targeting · retrovirus · adenovirus · liposome

THE IDENTIFICATION OF THE UNDERLYING genetic defects has recently made gene therapy an attractive treatment option for a wide variety of diseases. However, there is a corresponding requirement to produce vector systems that can deliver therapeutic genes to the appropriate target cells either in vivo or ex vivo. These systems must be both *efficient* and *accurate*. The range of different diseases amenable to intervention by gene therapy means, however, that no single delivery system is likely to be universally appropriate. For instance, the requirements of gene therapy for cystic fibrosis are greatly different from those of cancer. In the former case, only a certain proportion of a localized population of cells needs to be targeted with a single corrective gene; by contrast, cancer gene therapy usually involves the targeting of all of a diffusely spread population of cells, with the ultimate aim of killing rather than correcting them. Hence, the stringency with which the therapeutic gene needs to be accurately delivered can vary greatly. Expression of a copy of the cystic fibrosis transporter gene in nontarget cells is likely to be much less toxic than inadvertant expression of cytotoxic genes, aimed at cancer cells, but expressed in normal bystander cells.

Here, we review the progress in targeting gene delivery systems to specific target cell populations and look forward to the areas of research that will bring developments for the future. Unfortunately, improvements in the accuracy of a vector often compromise its efficiency, and vice versa. Nonetheless, it is clear that the technology now exists to incorporate specific targeting features into most of the currently available delivery systems. These may be at the level of *1)* target cell surface recognition, by manipulating the surface recognition components of viruses and liposomes; or *2)* target cell transcriptional restrictions, by incorporating transcriptional elements into plasmid or viral genomes such that the therapeutic gene is expressed only in certain target cell types.

The ultimate aim for the vectors of the future is to include these and other targeting opportunities within the same vehicle. In all probability, this will involve the incorporation of the most beneficial features of a variety of viral and nonviral systems into a single hybrid vector specifically custom built for each individual therapeutic situation.

TARGETING OF GENE THERAPY VECTORS AT THE LEVEL OF THE CELL SURFACE

Retroviral vectors

A primary determinant of retrovirus infectivity is the interaction between specific receptors on the host cell surface and glycoproteins (Env) on the lipid envelope of the retroviral particle. Ideally, targeted retroviral vectors for human gene therapy would use safe recombinant genomes and packaging lines from wild-type retroviruses that naturally display envelope proteins with the required tropisms. However, few naturally occurring retroviral infections are strictly limited to one cell type (1), and of the known receptors for retroviruses, only the HIV-1/SIV receptor CD-4 (2) is of relatively restricted distribution. Attempts have been made to produce vectors and packaging lines from HIV (3). However, HIV is a complex retrovirus that requires a number of self-encoded autoregulatory proteins, and this complicates the construction of stable packaging lines. Nevertheless, the principle of a recombinant HIV genome as a gene vector for $CD4^+$ cells has been demonstrated (3). However, vectors carrying HIV-1 *env* sequences would have to be used with extreme caution as the HIV-Env protein itself may be neurotoxic (4) or even immunosuppressive.

Most recombinant retroviral vectors and packaging lines produced so far have been based on murine leukemia viruses (MLVs)[2] (5). There are five recognized MLV groups (1) as

[1]To whom correspondence and reprint requests should be addressed, at: 14 Chemin des Aulx, 1228 Plan-les-ouates, Glaxo Institute of Molecular Biology, Geneva, Switzerland.

[2]Abbreviations: MLVs, murine leukemia viruses; MLV-E, ecotropic strain of MLV; MLV-A, amphotropic strain of MLV; RES, reticuloendothelial system; PEG, polyethylene glycol; ReSV, respiratory syncytial virus; ASOR, asialoorosomucoid; LCRs, locus control regions; DT-A, diphtheria toxin A; MVM, mouse minute virus.

0892-6638/95/0009-0190/$01.50.

defined by tropism, of which the most useful for gene delivery purposes have been the ecotropic strain (MLV-E), which infects virtually all rodent cells, and the amphotropic strain (MLV-A), which infects practically all mammalian cells. Packaging lines have therefore been created to allow production of retroviral vectors with host ranges that are either ecotropic or amphotropic, respectively (5). It is likely that all retroviral vectors suitable for human gene therapy in the near future will be based on such recombinant MLV genomes because they are well characterized with regard to safety and efficiency. For targeted retroviral vectors, then, the problem is either to restrict the promiscuous tropism of amphotropic particles or to confer upon ecotropic particles a limited human cell affinity. This could be done either by: *1)* genetic manipulation of the producer line such that ampho- or ecotropic Env is replaced by a different viral or nonviral protein having the required affinity; *2)* directly engineering a particular affinity into Env; or *3)* molecular conjugate approaches, in which ligands are coupled to the outside of the retroviral particle.

Replacement of Env: retroviral pseudotypes

The facility (5) with which *trans*- and *cis*-acting functions can be separated in MLV packaging lines allows easy experimental manipulation of the *trans*-acting function responsible for cellular tropism, namely, Env. This raises the possibility of replacing one viral *env* with that of another, thereby creating a hybrid producer line that generates "pseudotyped" viral vectors with a tropism conferred by the replacement *env* (**Fig. 1**). Phenotypic mixing has been used for many years as a tool to study receptor interactions (see ref 1 for a review); however, efforts have recently been directed at precisely replacing *env* and producing not envelope mixtures but vector populations exclusively displaying a novel tropism (1, 6). Such hybrid formation in general seems to occur more efficiently between closely related viruses. For instance, a recombinant MoMLV genome can be rescued by C-type viruses but not by HTLV-I or D-type viruses (7). However, provision of homologous or more closely related Gag proteins in some cases relaxes phenotypic restrictions on efficient pseudotyping of vector genomes with exogenous Env; for instance, an MoMLV vector can be packaged inside HTLV-1 (8) envelopes when MoMLV *gag-pol* are supplied in *trans*. Similarly, HIV has been given an extended host cell range by pseudotyping with the unrelated viruses HSV and VSV (9). Although these examples demonstrate the principle of creating an improved retroviral vector for human gene therapy by pseudotyping, so far they have produced only vectors with extended tropism rather than with restricted specificities.

The logical and necessary extension of pseudotyping approaches, then is to replace retroviral envelope genes with genes derived from nonviral sources. Although there are instances of nonviral glycoproteins being preferentially incorporated into retroviral particles, such as Thy-1 (10) and CD4 (11), actual infection of target cells, as opposed to specific binding, via display of such nonviral proteins has not been demonstrated, and is likely to require either fusogenic sequences within the foreign protein itself or coexpression of fusogenic molecules on the viral envelope.

Engineering Env

Genetic manipulations whereby sequences conferring specific binding affinities are engineered into preexisting viral *env* genes represent a promising approach. In MoMLV the sequences that determine receptor specificity seem to be in the most distal of the two variable regions within the amino-terminal portion of the SU Env subunit, and replacement of the variable region of one strain with that of another can, for instance, change viral tropism from that of strain

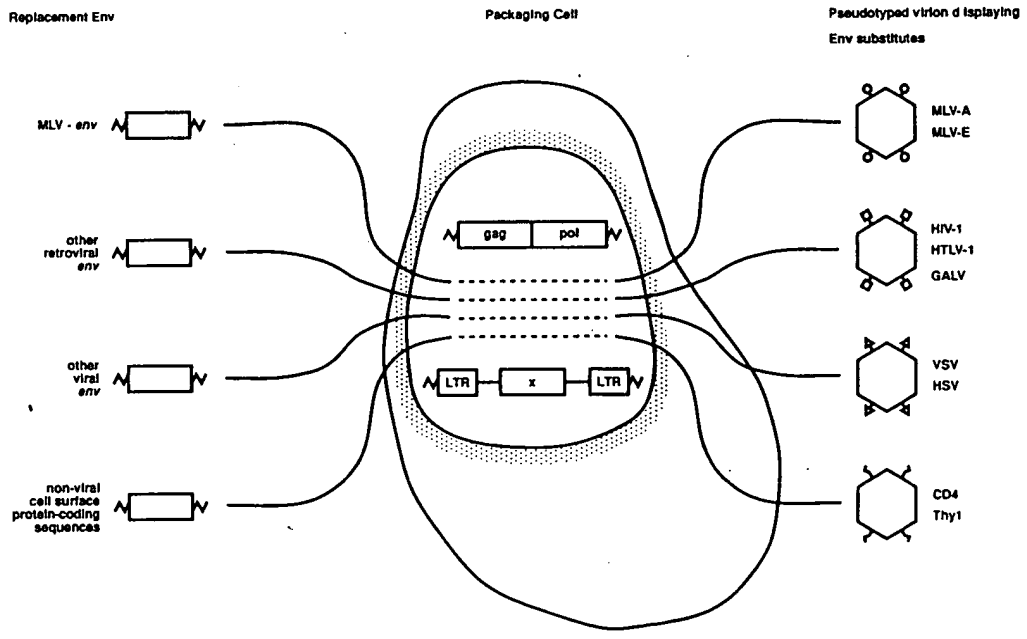


Figure 1. Generation of retroviral vectors with novel tropisms by construction of hybrid packaging lines. Transfection of a cell with genes (*gag-pol, env*) that encode viral *trans*-acting functions allows expression of all the structural components of the virion by that cell; these components can recognize and package the recombinant retroviral genome (shown here bounded by long terminal repeats (LTRs) and carrying a therapeutic gene x). Here we represent diagrammatically the various classes of retroviral pseudotypes that have been produced by providing various *env* genes in *trans;* this illustrates the principle of alteration of retroviral vector tropism by pseudotyping.

4070A to that of 10A1 (12). Engineering of murine retroviral Env proteins is being actively investigated (12-14) and is an important area of research. However, receptor recognition may involve complex interactions between the cellular ligand and different parts of the viral Env, and modification of viral tropism by direct replacement of receptor-binding sequences will not be straightforward. The function of Env proteins is not simply to adhere to host cells but also to participate in a sequence of events leading to membrane fusion. Excessive alteration of Env structure might therefore jeopardize the exposure of hydrophobic domains required for fusion and correct viral internalization. Nevertheless, a mammalian cell tropism has been conferred on an avian retrovirus by engineering integrin-binding sequences into Env. It was found that two of the variable regions of ALV Env could be manipulated by exchanging *env* sequences with those encoding a 16-amino acid RGD-containing peptide to produce Env proteins that were processed and incorporated into retroviral particles (15). Such hybrid envelopes could still efficiently mediate infection of avian cells through the ALV receptor, and could also infect and transfer neomycin resistance to mammalian (ALV-refractory) cells that expressed RGD-recognizing integrins. Infection was not efficient and required previous deglycosylation of the virus to expose RGD epitopes, but it is an important demonstration of the principle of targeting retroviral vectors by envelope modification.

In other studies, the RSV host range has been broadened to include human cells by packaging the genome with a chimeric Env that was a fusion of the RSV signal peptide and the influenza virus hemagglutinin (16). Chimeric Env was found to be incorporated into the virions as efficiently as wild-type RSV Env. It may be possible to use influenza hemagglutinins to direct retroviral vectors to subsets of cells exhibiting particular glycosylation phenotypes as the various influenza strains possess different hemagglutinins with different precise specificities. Another candidate protein for restriction of tropism is the B19 parvovirus surface protein, the surface receptor for which has recently been characterized (17) as the tetrasaccharide of globoside (blood group P antigen), which has a very limited tissue distribution. The B19 surface protein may be susceptible to fine-tuning of saccharide specificity by recombinant techniques or site-directed mutagenesis, similar to the influenza hemagglutinin (18).

The possibility of targeting retroviral vectors to particular glycosylation phenotypes may be of special interest for cancer therapy, as many transformed cells show altered glycosylation. Whether or not any aberrantly expressed glycans can mediate viral entry is another question; a recent report indicates that retroviruses targeted to cells via lectin cross-linking cannot infect the cells after binding (19), but this could be a function of the lectin or of structural alterations caused by cross-linking rather than a function of the glycan receptor.

The demonstrable ability (16) to alter RSV tropism from avian to human cells by manipulation of envelope structure could be of great interest for cancer therapy. This is because the vast number of target cells in malignant disease suggests that either the immune system must be recruited or that a replicating vector be used to target all the tumor cells, and RSV is a replicating vector par excellence. Besides its own genome, this virus is known to carry a cell-derived oncogene; replacement of this with a therapeutic cDNA would give a replication-competent gene therapy vector.

Encouraging results have been reported using a similar approach, in which a cDNA encoding an mAb fragment capable of hapten recognition was fused to the *env* gene of MoMLV (18). Coexpression of this gene with the normal envelope in an ecotropic packaging line resulted in infective viral particles that possessed the appropriate hapten-binding activity. It should be noted that the packaging line was expressing and required parental ecotropic Env as well as the chimeric protein, so it remains to be seen if infective retroviral particles can be assembled that contain only hapten-displaying Env (20). This approach has yet to be demonstrated using a hapten directed against a relevant human antigen capable of mediating virus internalization, and is still far from in vivo application.

Targeting by retrovirus-ligand conjugates

Hepatocytes possess a unique receptor that internalizes asialoglycoproteins. Conjugation of lactose to ecotropic viral particles allowed them to be recognized as asialoglycoproteins and broadened their host range to include human hepatoma cells (21). However, this approach is limited first to cells that express the asialoglycoprotein receptor, and second to proliferating cells (because retroviruses depend on host cell mitosis in order to integrate). As normal liver cells have a very low turnover rate, this technique is most likely to be of use for in vivo delivery to malignant liver disease of the hepatocyte lineage. Furthermore, because the vector was based on an ecotropic virus, its tropism in humans would be limited entirely to hepatocytes, greatly increasing its safety compared with broad affinity vectors such as those bearing the 4070A or GALV envelope proteins.

In a more indirect approach, it was found that ecotropic MoMLV vectors bound to human hepatoma cells after being cross-linked to the transferrin receptor by a series of antibodies; however, there was no subsequent proviral integration, suggesting either that the cross-linking antibodies were inhibiting membrane fusion or that the transferrin receptor cannot mediate appropriate viral internalization (22). A similar cross-linked mAb technique has been used to target ecotropic retroviral particles to human cells in vitro by means of the streptavidin-biotin reaction (23). This allowed ecotropic virus to bind to cells expressing human class I or II MHC antigens and to become internalized and integrated. An extension of this technique (19) showed that biotinylated EGF or insulin could substitute for the anticellular receptor antibody, and that EGF and insulin receptors could mediate internalization, leading to integration, of retroviral particles bearing streptavidin-conjugated antibodies. The possibility of targeting retroviral vectors by means other than murine antibodies, which suffer from numerous disadvantages in vivo, suggests that this approach may have potential although its in vivo applicability has yet to be demonstrated.

Adenoviral vectors

Adenoviruses are double-stranded DNA viruses in which the viral genomic DNA is contained in a virally encoded protein coat (capsid) rather than a phospholipid bilayer of host cell origin. The capsid consists of three major types of subunit: the hexon, which makes up the bulk of the coat; the penton base; and the penton fiber. The fiber is attached to the capsid via the penton base and projects outward; base and fiber together are known as the penton complex. During infection, the fiber mediates initial binding of the virus to an unidentified cellular receptor and the penton base subsequently mediates virus internalization via interactions with α_v-type integrins (24). Thus, the penton complex is respon-

sible for binding and internalization, and therefore for viral tropism at the level of cell recognition. Although adenoviral diseases are usually associated in vivo with respiratory epithelium or the GI tract, their cellular receptors seem to be widely distributed (25). Clearly then, as with retroviruses, the problem is to limit viral tropism to a particular subset of tissues. The adenoviral proteins responsible for attachment and internalization, respectively, have been well characterized, giving two points at which to manipulate tropism. The most promising approach is to restrict adenovirus infection at the cell-binding stage by replacing the carboxyl-terminus knob of the fiber with a ligand conferring a particular tropism, for instance, with an antibody hapten. One report (26) describes the restriction of adenovirus type 5 tropism by a different kind of fiber modification where intact virions were chemically modified so that their fiber carbohydrate groups were covalently linked to an asialoglycoprotein-polylysine conjugate. Such modified virus was found to have much decreased infectivity to asialoglycoprotein receptor-negative cells while retaining infectivity to receptor-positive cells. This approach would be equally applicable to targeting adenoviral vectors per se. It may also be possible to restrict infection by replacing the RGD-containing domain of the penton base with sequences having affinity for a ligand other than RGD-recognizing integrins.

Adenoviral vectors can also be targeted via the route of administration (27); targeting of a *lacZ*-expressing adenoviral vector to the kidney by renal artery or pyelic cavity infusion resulted in β-gal activity in various renal cells with no detectable expression in liver, lung, or bladder cells (27).

A possible advantage of refinement of vector targeting to the point of absolute specificity might be the ability to use replicating vectors for gene therapy. For cancer, development of a replicating adenoviral vector, perhaps carrying a cytokine or suicide gene, targeted to cancer cells at the level of cell binding (via fiber/base manipulations) and at the level of transcription (see next section) might allow transduction of the large number of malignant cells in a tumor deposit; cell death due to adenovirally induced lysis may even potentiate the field effect of cytokines. A safety feature of such a system would be that the immune system would be expected to eventually clear such therapeutic infections (as it does for wild-type infections); therefore this potential therapy only awaits adequate targeting strategies.

Liposome vectors

Most work on targeted liposomes has been designed to deliver cytotoxic drugs to cancer cells and has been reviewed recently (28). Expression of a cDNA in the target cells makes greater demands on the vector system in that it must not only target the appropriate cell type but also allow efficient delivery of undegraded DNA to the nucleus. For most targeted gene delivery purposes, conventional liposomes are limited because of their selective uptake by cells of the reticuloendothelial system (RES), in particular by macrophages resident in liver, spleen, and bone marrow, because of their limited extent of extravasation. Where macrophages themselves are the target, however, RES affinity is advantageous. In *L. donovani* leishmaniasis parasites not only multiply in the Kupffer cells of the liver, but are also resident in a vacuole to which lysosomes fuse, so that liposomes are passively targeted not only to the parasitized cell but also to the appropriate organelle, making liposome-mediated delivery of transcriptionally targeted antisense or suicide genes to these parasites a real possibility. It is also possible in a few cases to avoid much of the RES by the particular route of application, particularly where the target tissue is found in a discrete anatomical compartment; e.g., nontargeted liposomes could be applied directly to the bladder for treatment of carcinoma or to the lung for treatment of cystic fibrosis or αAT deficiency. Targeting by compartment has allowed confined transduction of discrete sections of arterial wall using both liposomal and retroviral vectors (29).

In most cases, however, in vivo use of liposomes requires first avoiding the RES, and second, display of appropriate tropic and fusogenic molecules (**Fig. 2**). Uptake by the RES can be considerably delayed, but not altogether avoided, by the use of "stealth" liposomes that display negatively charged moieties such as the ganglioside GM1 and polyethylene glycol (PEG) (28). For most systemic purposes, the stealth formula is probably essential.

Liposomes bearing an immunoglobulin complement ("immunoliposomes") can exhibit tropisms conferred by the displayed antibody. Hence, coupling to liposomes of an antibody against glioma cells increased the efficiency of gene delivery to these cells in culture by about sevenfold (30). Just as mAbs may be conjugated to liposomes to confer targeting capability, so may other ligands such as growth factors and hormones. Coupling of transferrin to liposomes followed by i.v. injection in a rabbit model resulted in significantly greater localization to bone marrow erythroblasts (31), and incorporation of surfactant protein A into liposomes increased the uptake of the liposome cargo by alveolar type II cells (32). However, it is not sufficient merely to confer upon the vector a particular binding ability; the particle must bind to a ligand that also allows fusion of liposome and cell membranes. Such considerations of appropriate internalization of vector cargo are especially important for gene delivery vectors, where the DNA must not only reach the appropriate cell type but also must reach the nucleus in undegraded form.

Conjugating virions to liposomes or incorporating viral surface glycoproteins into liposomes might create a vector that has the efficient cell attachment and entry mechanisms of a virus but not the safety drawbacks; much work has been done in this area with Sendai virus in particular (33). Another system used liposomes that displayed only the fusogenic protein of Sendai virus (F-protein) and not the cell-binding protein (hemagglutinin) (34). However, although

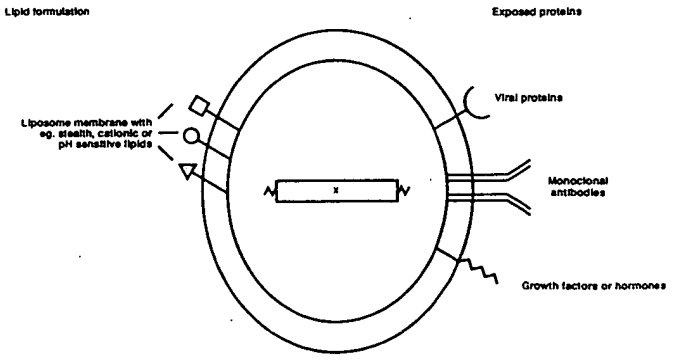


Figure 2. Modification of lipid membranes to produce targeted liposomes. Targeting of liposomes requires first abrogation of their RES affinity, and second, provision with exposed ligands having the required targeting capacity. Inclusion of ganglioside glycolipids into the lipid formulation can allow RES evasion; other lipid formulations include cationic lipids to allow promiscuous membrane binding and hence lysosome escape, and pH-sensitive lipids, which allow lysosome escape without the broad affinity conferred by cationic lipids. Various types of ligand can be inserted into the lipid membrane for provision of particular tropisms (see text for details).

such approaches can make liposomes up to 10-fold more efficient than lipofection at gene delivery (33), in terms of targeting all it can do is confer upon the liposome the tropism of the virus, and there are very few native viral receptors that exhibit a narrow and precise cell type specificity. Nevertheless, a promising system (35) is currently being developed in which respiratory epithelium is targeted by means of the surface proteins of respiratory syncytial virus (ReSV), which is responsible for infections of the lower respiratory tract. Liposome-type envelopes were constructed that displayed both the attachment and fusion proteins of ReSV, and these have been shown to enter all cells of a cultured respiratory epithelial cell line within 1 h (35).

Cationic liposomes such as the commercially produced lipofectin can efficiently avoid the lysosomal pathway because the particular lipid composition allows direct fusion of liposome and cell membranes. These particles are therefore much more efficient than conventional liposomes, and for in vitro transduction have largely replaced them. Cationic liposomes have also been used for in vivo approaches and even clinical trials; however, there seem to be no data on the extent to which these liposomes can avoid the RES, and indeed the cationic surface would seem to be incompatible with the negative charges characteristic of the stealth formulation. One report suggests that the cationic liposome has as much affinity for other cell types as for the RES after i.v. injection (36). Administration of liposomes carrying SV40-CAT resulted in widespread expression of the marker gene for up to 9 wk, albeit mainly in tissues generally associated with the RES such as spleen, liver, lymph nodes, and bone marrow as well as in vascular endothelium. CAT expression was also observed in tumor cells in this experiment, probably as a consequence of leaky tumor vasculature. It may eventually be possible to combine the efficient lysosomal avoidance of cationic liposomes with a specific targeting capacity, although the problem is likely to be that the generally fusogenic nature of cationic liposomes may preclude any precisely restricted targeting.

Molecular conjugate vectors

Targeting of plasmid DNA may be achieved by coupling the DNA to a ligand with a demonstrated cell or tissue affinity. This is usually brought about by covalently linking a polycation such as polylysine to the ligand; the polycation can then bind to and condense plasmid DNA via electrostatic interactions, leaving the ligand exposed on the surface of the conjugate (37). The ligands chosen must be efficiently endocytosed in the target cells so that DNA is efficiently internalized. One of the first receptors to be used in this way was the asialoglycoprotein receptor, whose expression is limited to hepatocytes; this receptor binds glycoproteins with terminal galactose residues for removal from the circulation; asialoorosomucoid (ASOR) is a major natural ligand for this receptor. BSA has been given specificity for the ASOR receptor by artificial galactosylation, and has been used to target CAT and human factor IX cDNAs (38) to hepatoma cells in vitro and to liver but not other tissues in vivo. Other ligands that have been used in similar conjugates include insulin (39), EGF (40), lectins (41), and transferrin (37). A major drawback of classical molecular conjugate vectors is that internalization depends on receptor-mediated endocytosis, a process that directs the receptor complex to lysosomes where it is degraded; only a small fraction of introduced

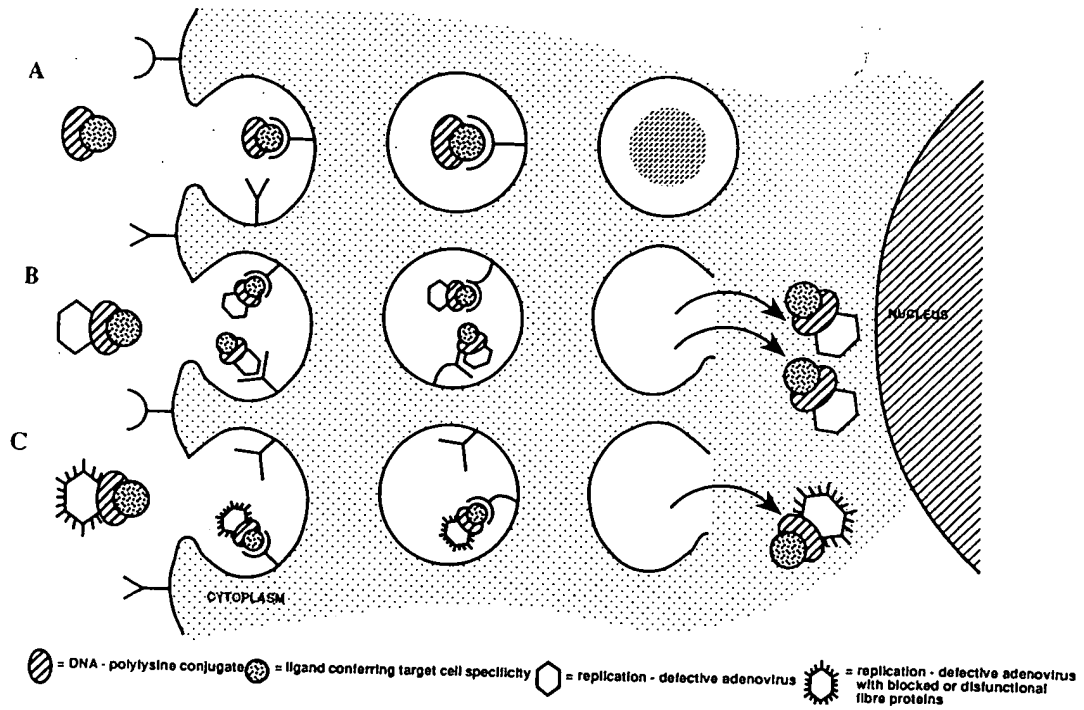


Figure 3. Targeting of plasmid DNA by molecular conjugate vectors. Conjugation of plasmid DNA to a particular ligand can confer a particular targeting capacity, but results in a vector of very low efficiency because most receptor-mediated endocytosis directs such conjugates to lysosomes where the great majority of vector DNA is degraded (route A). By complexing an adenovirus coat to the conjugate, a highly efficient vector is created by virtue of the ability of adenovirus proteins to disrupt the endosome before vector degradation (route B); however, this abrogates any targeting capacity conferred by the ligand, as the complex can enter cells either via the ligand receptor or via the virtually ubiquitous adenoviral receptor. To truly target such complexes it will be necessary to use modified adenoviral coats that retain the lysosomal escape mechanism but cannot interact with the adenoviral receptor (route C).

DNA escapes this pathway and enters the nucleus, leading to low efficiency of transduction.

A new generation of molecular conjugate vectors has been produced that has the capacity to escape the degradative lysosomal pathway by utilizing features of the adenovirus capsid (**Fig. 3**). Adenovirus disrupts endosomes during cell entry as a consequence of a conformational change in the capsid proteins, resulting in membrane breakdown, triggered by a drop in pH. Hence, molecular conjugate vectors delivered DNA to cells with greatly increased efficiency when transfection was done in the presence of adenovirus. However, this effect relies on both virus and vector being present in the same endosome. To improve efficiency, the adenovirus has been coupled directly to the molecular conjugate (37). However, adenovirus receptors are virtually ubiquitous and so the coupling of an adenovirus receptor to a targeted molecular conjugate would be expected to partially or completely abrogate any preferential tropism conferred by the ligand. Blocking the interaction of fiber with adenovirus receptor by mAb to the fiber resulted (42) in a vector that was both targeted to a specific subset of cells and able to escape the lysosomal pathway. A more satisfactory approach would be to create recombinant adenoviral vectors that display dysfunctional fiber proteins in order to bypass the antibody-coating step.

Few in vivo experiments have been attempted using adenovirus-molecular conjugate complexes, and in fact it is unlikely that such vectors will be routinely applicable to in vivo work, although they are likely to be of use for ex vivo strategies (43). This is a consequence first of the size of the complex (transferrin-polycation conjugates are approximately 100 nm in diameter (44); complexed with AdV they would be even larger), which will prohibit extensive extravasation or tissue penetration, and second, of the likelihood of direct immunogenicity of the AdV proteins (45).

TARGETING OF GENE THERAPY VECTORS AT THE GENETIC LEVEL

Transcriptional targeting

Therapeutic cDNAs may be limited in expression to a particular subset of cells by placing them under the control of regulatory elements that possess binding sites for tissue-restricted positive or negative *trans*-acting factors (**Fig. 4**). Correctly regulated expression may require, in addition to 5' promoter sequences, distant elements either 5' or 3' to the coding region; these elements act together with the promoter and allow tissue-specific expression at appropriate levels independent of position of integration. Such locus control regions (LCRs) have been identified for a number of genes. LCRs would be of much use for gene augmentation but the transfer of such large sections of DNA to target cells will be problematic, particularly in vivo, and in fact for the foreseeable future may be confined to ex vivo strategies. Where a monogenic defect results in pathology in more than one tissue, the most pragmatic approach to appropriately limit the expression of therapeutic cDNA is to use the cellular promoter/enhancer elements native to the defective gene. Furthermore, the use of cellular rather than viral promoters reduces the chance of loss of cDNA expression due to inactivation of viral sequences by methylation or other mechanisms (46). Thus, cellular promoters may confer benefits both of long-term expression and of tissue-restricted expression, and where vector-targeting at the cell-binding level has not been achieved it may represent the only way of limiting expression of exogenous cDNA.

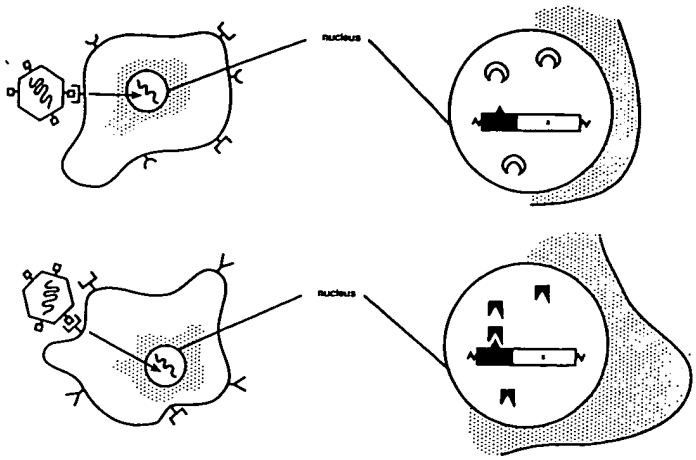


Figure 4. Tissue-restricted transcription. A promiscuously binding vector can be targeted at the transcriptional level if the therapeutic gene (x) is controlled by 5' regulatory elements (shown here as a shaded region upstream of x) active only in the presence of tissue-specific nuclear transcription factors; thus expression of x occurs only in the target cells.

Tissue-specific cellular regulatory elements have great potential for development of safe, targeted vectors for gene therapy. For example, the creatine kinase promoter has been used in a plasmid vector to restrict dystrophin cDNA expression to skeletal and cardiac muscle, and in the *mdx* mouse model of Duchenne muscular dystrophy, mice transgenic for this promoter-cDNA construct were found to exhibit correction of dystrophic symptoms (47). A potential approach to the treatment of B cell lymphoma involves expression of suicide genes transcriptionally regulated by promoter/enhancers from the Ig heavy chain or the κ light chain genes; expression plasmids containing the diphtheria toxin A (DT-A) gene controlled by these regulatory elements mediated significant expression of DT-A in B lymphoid cells but not in HeLa cells or fibroblasts (48).

Endothelial cells are attractive recipients for gene transfer therapies not only for obvious purposes such as targeting of tumor vasculature or therapy of cardiovascular disease, but also for the systemic secretion of therapeutic factors. An endothelial cell-specific regulatory region has recently been characterized (49) as 500 bp of 5' sequences, associated with the gene for von Willebrand's factor, acting in conjunction with an essential region in the first intron. This promoter could be particularly useful when driving a suicide gene in a retroviral vector as it would then be targeted to dividing endothelial cells, i.e., almost exclusively tumor vasculature.

Tissue-specific cellular promoters frequently retain their specificity in the context of a retroviral vector (50); however, this is not always the case, and the design of the retroviral vector may have significant effects on tissue specificity due to promoter interference (51). Tissue-specific promoters have also been shown to appropriately restrict cDNA expression in the context of recombinant adenoviruses, e.g., the rat albumin promoter maintained its hepatoma cell specificity in vitro (52), albeit at low levels.

Antiviral therapy using transcriptional targeting

Transcriptional targeting may be of particular use in the therapy of particular kinds of viral infection. In cases where the viral life cycle depends on self-encoded autoregulatory

proteins, vectors can be made in which therapeutic cDNAs are transcriptionally regulated by these same viral proteins. Transcription of the therapeutic cDNA is therefore limited to cells that are infected by the virus, and thus such an approach could be either prophylactic or curative. This strategy has been applied to experimental HIV therapies. One recent report (53) described the construction of a recombinant retrovirus containing HSV-TK driven by the HIV-2 LTR-TAR; cells expressing this construct became susceptible to ganciclovir after infection by HIV-2 in vitro.

Targeting proliferating cells

Murine C-type retroviral vectors can combine the ability to express cDNA from an internal tissue-specific promoter with an innate tropism for proliferating tissue. Therefore, they have great potential as vectors for the gene therapy of cancer, because restricted cDNA expression is of particular importance in strategies that involve delivery of cytokine or suicide genes and malignancies are often distinguished by rapid division in a relatively quiescent background. Indeed, in a very few cases the retroviral requirement for cell division may be sufficient in itself to target the therapy (**Fig. 5**); where tumors arise in the CNS their high rate of proliferation in the context of a completely postmitotic tissue, in an anatomical compartment that is separated from the rest of the body, allows efficient targeting with retroviral vectors (54). As an additional targeting feature for malignancies of the CNS, the glial-specific promoter region of the mouse myelin basic protein gene has been used to drive HSV-TK in a retroviral vector (55); this approach could allow long-term administration of producer cells at the primary site or systemic vector application to treat metastatic deposits as collateral infection of nonglial cells would not result in expression of the suicide gene.

Retroviral vectors would also be useful in targeting liver malignancies, as the liver is also slowly proliferative under normal circumstances. Tissue-specific promoters would be essential for such strategies, because unlike the CNS, the liver is not efficiently insulated from the rest of the body. Amphotropic retroviral vectors have been constructed carrying HSV-TK cDNA driven either by the albumin or the a-fetoprotein promoters (56). The albumin promoter was active only in cells of the liver lineage; the a-fetoprotein promoter conferred an extra level of targeting in that it was hepatoma-specific as opposed to hepatocyte-specific (a-fetoprotein is normally expressed only in fetal tissues).

The 5' region of the tyrosinase gene has also been used to restrict expression of therapeutic cDNAs to melanocytes and melanoma cells both in vitro and in vivo by means of retroviral vectors (51, 57). This kind of transcriptional targeting may be useful in VDEPT approaches for melanoma because normal melanocytes are dispersed and of low density in body tissues, and their ablation is likely to be minimally pathological. Even better would be the usurpation of tumor-specific transcriptional regulation by using promoter sequences from genes whose overexpression is limited to transformed tissue. One such candidate is the oncogene ERBB2, which is overexpressed in a variety of tumors. The ERBB2 promoter sequences have been used to drive cytosine deaminase cDNA in a retroviral vector (58); this strategy conferred sensitivity to ERBB2-overproducing cells but not to control cells, and represents a potentially widely applicable method of tumor-preferential transcriptional targeting. The a-fetoprotein promoter is in effect completely tumor-specific, but is applicable only to malignancies of the liver.

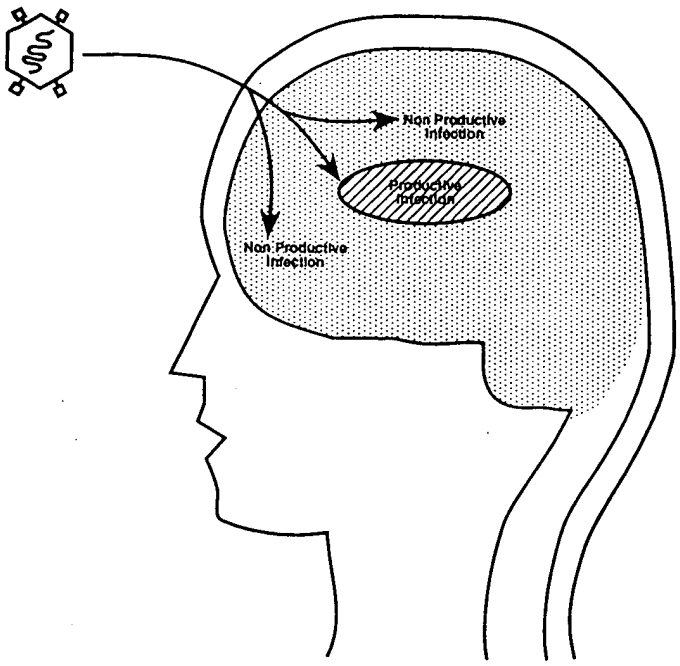


Figure 5. Targeting proliferating cells. Retroviral vectors require cell division for integration and gene expression; therefore where a tumor arises in a completely postmitotic background, such as the CNS, the proliferation of the malignant tissue may be sufficient in itself to allow efficiently targeted delivery of suicide genes via recombinant retroviruses. Actively replicating (tumor) cells are represented by diagonal lines; quiescent neuron tissue is represented by dots.

Exploitation of natural viral tropisms

An obvious approach to the precise targeting of tissues is to make vectors from viruses that have preferential patterns of transcription in target tissues, such as HSV vectors for nervous tissue. However, careful dissection of the genomes of these viruses will be necessary to separate pathogenic sequences from those that confer transcriptional specificity; in most cases it will be preferable to use cellular promoters in the vector of choice, especially as the range of transcriptionally targeted viral genomes is not great.

There may be one remarkable exception to the general requirement for cellular promoters rather than viral promoters in gene therapy, namely, the use of autonomous parvoviral sequences for targeting transformed cells (see ref 59 for review). These viruses preferentially kill transformed cells (60), and coinjection of mouse minute virus (MVM) and Ehrlich ascites tumor cells into the peritoneal cavities of mice inhibited tumor formation by up to 90%. Furthermore, mice that had survived one such coinjection were resistant to a second tumor challenge 5-6 wk later. The precise basis of parvovirus oncotropism is not understood but may be related to an effect of the transformed cell environment on the production or activity of parvovirus autoregulatory proteins. The parvovirus promoter that is preferentially transactivated in certain transformed cells is clearly a candidate to control transcription of suicide or cytokine genes in parvovirus vectors for cancer therapies. Recombinant parvovirus vectors have been made and shown to both transfer exogenous cDNA expression to recipient cells and retain their oncotropism in vitro (61) for human and murine cells. Recombinant parvoviruses may therefore represent one of the most promising approaches to cancer therapies for the future.

Targeted integration: site-specific recombination

Nonintegrating vectors are adequate for transient expression of cDNA. Where the object is a "one-shot" treatment for cure of a genetic disorder, it is necessary to use either an integrating vector or a stably replicating extrachromosomal element. For the future, sequences containing mammalian origins of replication or even entire mammalian artificial chromosomes (62) could have great potential especially for ex vivo approaches. Similarly, vectors based on the Epstein-Barr virus, which is stably maintained episomally as a plasmid in human cells, may one day be suitable for clinical use.

The ideal approach would be to target the exogenous DNA to the mutant gene, i.e., gene replacement rather than gene augmentation. Such gene targeting approaches may be of use for ex vivo strategies to stably transduce cells with less likelihood of simultaneous transformation (63). Such in vitro homologous recombination may be useful in inactivating genes responsible for MHC class I expression in myoblasts to create a universal carrier cell that can be transplanted regardless of the recipient HLA type (63). This approach is applicable to any ex vivo strategy that requires implantation of viable transduced but otherwise unchanged cells. The technology required to accomplish this at levels of efficiency relevant to in vivo gene transfer does not yet exist and so integrating gene therapy vectors at present can offer only gene augmentation.

Nontargeted integration could be hazardous if completely random, not only by turning on downstream oncogenes via promoter readthrough but also by direct disruption of genes, and this is the main source of concern with regard to the use of retroviral vectors in humans. Vectors with the capacity for site-specific integration would overcome these problems. Adeno-associated virus is a defective parvovirus that potentially is widely applicable in gene transfer strategies because it is tropic for many cell types, nonpathogenic in humans (in the absence of helper virus the AAV genome does not replicate but integrates into the genome and assumes a state of latency), and can be manipulated to derive recombinant genomes capable of vectoring exogenous DNA (64). Although these vectors can package only up to 4.5 kb as compared with the retrovirus limit of approximately 7 kb, they are said to have one major advantage over other integrating vectors, namely, a propensity (which is far from total) for apparently harmless integration into a region of human chromosome 19 known as AAVS1 (see review, ref 65). Where such specific integration occurs, it is almost certainly mediated by virally encoded proteins with affinity both for the target site and for the virus genome (66). Although integrated viral sequences remain dormant until superinfection by AdV/HSV, exogenous cDNAs driven by internal promoters can still be active (furthermore, the transcriptional inactivity of the viral ITR means that there will be no promoter interference leading to, for example, loss of tissue specificity of exogenous promoter, and less chance of insertional mutagenesis for the same reason). Thus AAV vectors have been shown to confer neomycin resistance and in some cases to integrate with site specificity (64). This study also showed that AAV vectors preserved their site specificity after transfection in plasmid form; the use of a transfectable plasmid rather than a viral vector might overcome the packaging limitations of AAV vectors (64). It must be said, however, that some groups report that recombinant AAV vectors show site specificity in only a relatively minor proportion of the total number of integration events. There have been several attempts to explore the therapeutic potential of AAV vectors, e.g., the delivery of cDNA for the correction of the cystic fibrosis defects (67).

There may be other vector systems also capable of site-specific integration. Eukaryotic genomes harbor large numbers of endogenous transposable elements of various types (68), i.e., autonomously replicating units that can insert themselves into the host genome. Some of these elements, known as LTR retrotransposons, are very similar to retroviruses both in replication cycle and in organization, being bound by LTRs and possessing coding regions with homology to retroviral *gag-pol* genes. The replicative cycle of LTR retrotransposons exactly parallels that of the retroviruses except that there is no envelope stage, thus, cytoplasmic virus-like particles (69) are formed containing reverse transcriptase, the RNA form of the retrotransposon, and cellular tRNA primers for reverse transcription. Such elements include *copia*, yeast Ty, and the intracisternal A particle of mice; clearly they have great potential as vectors of improved safety as their use with retroviral packaging lines would be less likely to result in helper virus production through homologous recombination. Indeed a mouse retrotransposon VL30 has already been made into a gene transfer vector (70), which can be produced in a standard retroviral packaging line. Endogenous retrotransposons a priori would be expected, through coevolution with the host genome, to display a degree of site specificity of integration as continuous random retrotransposition would be deleterious to the cell. Yeast retrotransposons offer the best examples of site-specific retrotransposons, and moreover, their site of integration appears to be benign. Two of the five *Saccharomyces cerevisiae* retrotransposons, Ty1 and Ty3, exhibit unambiguous site specificity of integration (71). Ty3 elements integrate into sites upstream of genes transcribed by RNA pol III, frequently within 1-4 nucleotides of the start site of transcription. It has been suggested that this sequence-independent site specificity is brought about by interaction of the retrotransposon with elements involved in RNA pol III-mediated transcription, e.g., TFIIIB (71). Similarly, Ty1 preferentially integrates upstream of tRNA genes (71) 57% of insertions occurring within 400 bp of a tRNA gene. A consequence of this specificity is that yeast genes are only rarely interrupted by Ty1 insertions as regions upstream of yeast tRNA genes rarely contain open reading frames (71). The great similarity of LTR retrotransposons to retroviruses allows them to be made into vectors with conventional retrovirus packaging lines (70); possibly the development of a packaging line that provides retrotransposon rather than retroviral *gag-pol* in *trans* will allow the production of vectors with integrational site specificity.

SUMMARY AND PERSPECTIVES

Of the gene therapy protocols that have so far entered clinical trials, targeting of the appropriate vectors has been achieved largely only by indirect means. Thus, several such trials (for example, for treatment of ADA deficiency, HIV infection, or cancer) have used specific cell populations that have been removed from the patient and infected in vitro by nontargeted amphotropic retroviruses before being returned in vivo. Further levels of targeting have been achieved in some cases by careful choice of the patient's cells; for instance, ex vivo transduction of tumor infiltrating lymphocytes with potentially tumoricidal genes has been proposed as a means of delivering their products to tumor deposits at much higher concentrations than would otherwise be possible.

In contrast to ex vivo manipulation of target cells where the vector requires very little, if any, intrinsic targeting capability, there are an increasing number of protocols in which

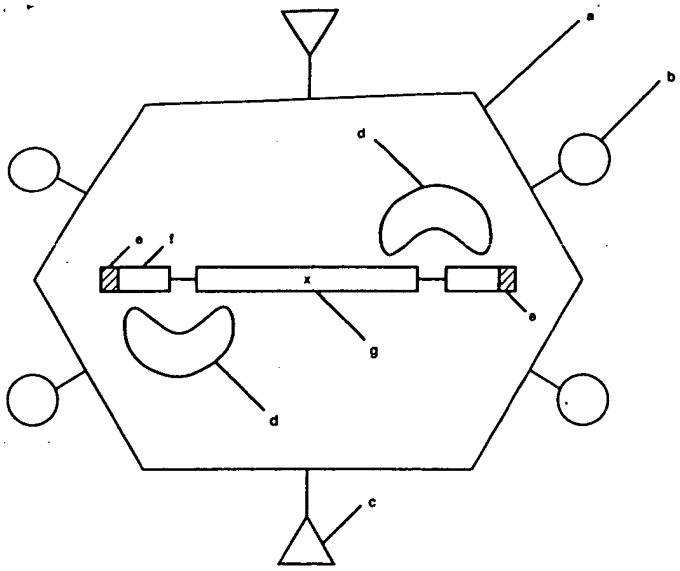


Figure 6. A theoretical composite vector. Some features that might be incorporated in an ideal synthetic vector include a stable, nonimmunogenic envelope, probably lipid (*a*); exposed ligands to confer a particular affinity on the vector (*b*); moieties that encourage fusion between vector and target cell membranes (*c*); proteins to allow directed integration of vector DNA, e.g., site-specific recombinases (*d*); sequences to enable homologous recombination between vector DNA and particular loci of the target genome (*e*); tissue-specific promoter regions to allow restricted expression of the therapeutic gene (*f*); and the therapeutic cDNA (*g*).

recombinant genes are delivered directly to patients in vivo (such as for the treatment of cystic fibrosis and cancer). Once again, targeting at the level of the vector has not yet been particularly well developed; hence, liposome or viral-mediated delivery of the CFTR gene to airway epithelial cells of CF patients has relied largely on the localized delivery of the vectors directly to the affected tissues, and on the fact that there is good evidence that inadvertant expression of the CFTR gene in cells other than the target epithelial cells may have few adverse effects. Localized delivery has also been used in the treatment of brain tumor deposits, using stereotactic injection of retroviral producer cells, but with the added sophistication that the retroviruses would be expected to infect only the actively dividing tumor cells and not the surrounding neural tissue.

However, for the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances in the ability with which clinicians can confidently administer recombinant vectors for the treatment of genetic disease directly to affected tissues in vivo. For this to occur, many targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems. Vectors have already been developed that incorporate transcriptional specificity for a certain tissue type; however, the development of surface targeting has been more problematic in most cases. The biggest challenge for the next 5 years will be to combine targeting with efficiency in the production of the vector systems of the future. So far, attainment of one usually compromises the other; for example, we have constructed retroviral vectors targeted at the level of transcription to melanoma cells but these viruses are generally of lower titer than their nontargeted counterparts.

Nonetheless, the imagination and the technology is currently available to allow us to hope that vectors will eventually be constructed that can include both efficiency and specificity. In particular, it does not seem unrealistic to suppose that the gene therapy vectors of the future will not be based exclusively on any single virus or physical vector system alone but will be synthetic, custom-designed vehicles (**Fig. 6**) into which specific targeting features can be included depending on the particular clinical requirements of the target disease and tissue. FJ

We would like to thank Professor Bob Williamson for influential discussions.

REFERENCES

1. Weiss, R. A. (1993) Cellular receptors and viral glycoproteins involved in retrovirus entry. In *The Retroviridae* (Levy, J. A., ed) pp. 1-108, Plenum, New York
2. Dalgleish, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F., and Weiss, R. A. (1984) The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312**, 763
3. Poznansky, M., Lever, A., Bergeron, L., Haseltine, W., and Sodroski, J. (1991) Gene transfer into human lymphocytes by a defective human immunodeficiency virus type 1 vector. *J. Virol.* **65**, 532-536
4. Toggas, S. M., Masliah, E., Rockenstein, E. M., Rall, G. F., Abraham, C. R., and Mucke, L. (1994) Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. *Nature* **367**, 188-193
5. Miller, A. D. (1992) Retroviral vectors. *Curr. Top. Microbiol. Immunol.* **158**, 1-24
6. Landau, N. R., Page, K. A., and Littman, D. R. (1991) Pseudotyping with human T-cell leukemia virus type I broadens the human immunodeficiency virus host range. *J. Virol.* **65**, 162-169
7. Takeuchi, Y., Simpson, G., Vile, R. G., Weiss, R. A., and Collins, M. K. L. (1992) Retroviral pseudotypes produced by rescue of a Moloney murine leukemia virus vector by C-type, but not D-type, retroviruses. *Virology* **186**, 792-794
8. Vile, R. G., Schulz, T. F., Danos, O. F., Collins, M. K. L., and Weiss, R. A. (1991) A murine cell line producing HTLV-I pseudotype virions carrying a selectable marker gene. *Virology* **180**, 420-424
9. Zhu, Z., Chen, S. S. L., and Huang, A. S. (1990) Phenotypic mixing between human immunodeficiency virus and vesicular stomatitis virus or herpes simplex virus. *J. AIDS* **3**, 215-219
10. Calafat, J., Janssen, H., Demant, P., Hilgers, J., and Zavada, J. (1983) Specific selection of host cell glycoproteins during assembly of murine leukaemia virus and vesicular stomatitis virus. *J. Gen. Virol.* **64**, 1241-1253
11. Young, J. A. T., Bates, P., Willert, K., and Varmus, H. E. (1990) Efficient incorporation of human CD4 protein into avian leukosis virus particles. *Science* **250**, 1421-1423
12. Ott, D., and Rein, A. (1992) Basis for receptor specificity of nonecotropic murine leukemia virus surface glycoprotein $gp70^{SU}$. *J. Virol.* **66**, 4632-4638
13. Battini, J.-L., Heard, J. M., and Danos, O. (1992) Receptor choice determinants in the envelope glycoproteins of amphotropic, xenotropic and polytropic murine leukemia viruses. *J. Virol.* **66**, 1468-1475
14. Morgan, R. A., Nussbaum, O., Muenchau, D. D., Shu, L., Couture, L., and French Anderson, W. (1993) Analysis of the functional and host range-determining regions of the murine ecotropic and amphotropic retrovirus envelope proteins. *J. Virol.* **67**, 4712-4721
15. Valsesia-Wittman, S., Drynda, A., Deleage, G., Aumailley, M., Heard, J.-M., Danos, O., Verdier, G., and Cosset, F.-L. (1994) Modifications in the binding domain of avian retrovirus envelope protein to redirect the host range of retroviral vectors. *J. Virol.* **68**, 4609-4619
16. Dong, J., Roth, M. G., and Hunter, E. (1992) A chimeric avian retrovirus containing the influenza virus hemagglutinin gene has an expanded host range. *J. Virol.* **66**, 7374-7382
17. Brown, K. E., Anderson, S. M., and Young, N. S. (1993) Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* **262**, 114-117
18. Rogers, G. N., Paulson, J. C., Daniels, R. S., Skehel, J. J., Wilson, I. A., and Wiley, D. C. (1983) Single amino acid substitutions in influenza haemagglutinin change receptor-binding specificity. *Nature* **304**, 76-78
19. Etienne-Julan, M., Roux, P., Carillo, S., Jeanteur, P., and Piechaczyk, M. (2222) The efficiency of cell targeting by recombinant retroviruses depends on the nature of the receptor and the composition of the artificial cell-virus linker. *J. Gen. Virol.* **73**, 3251-3255
20. Russell, S. J., Hawkins, R. E., and Winter, G. (1993) Retroviral vectors displaying functional antibody fragments. *NAR* **21**, 1081-1085
21. Neda, H., Wu, C. H., and Wu, G. Y. (1991) Chemical modification of an ecotropic murine leukemia virus results in redirection of its target cell specificity. *J. Biol. Chem.* **266**, 14143-14146

22. Goud, B., Legrain, P., and Buttin, G. (1988) Antibody-mediated binding of a murine ecotropic Moloney retroviral vector to human cells allows internalisation but not the establishment of the proviral state. *Virology* **163**, 251-254
23. Roux, P., Jeanteur, P., and Piechaczyk, M. (1989) A versatile and potentially general approach to the targeting of specific cell types by retroviruses: application to the infection of human cells by means of major histocompatibility complex class I and class II antigens by mouse ecotropic murine leukemia virus-derived viruses. *Proc. Natl. Acad. Sci. USA* **86**, 9079-9083
24. Wickham, T. J., Mathias, P., Cheresh, D. A., and Nemerow, G. R. (1993) Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ promote adenovirus internalisation but not virus attachment. *Cell* **73**, 309-319
25. Stratford-Perricaudet, L. D., Makeh, I., Perricaudet, M., and Briand, P. (1992) Widespread long-term gene transfer to mouse skeletal muscles and heart. *J. Clin. Invest.* **90**, 626-6330
26. Wu, G. Y., Zhan, P., Sze, L. L., Rosenberg, A. R., and Wu, C. H. (1994) Incorporation of adenovirus into a ligand-based DNA carrier system results in retention of original receptor specificity and enhances targeted gene expression. *J. Biol. Chem.* **269**, 11542-11546
27. Moullier, P., Friedlander, G., Calise, D., Ronco, P., Perricaudet, M., and Ferry, N. (1994) Adenoviral-mediated gene transfer to renal tubular cells in vivo. *Kidney Int.* **45**, 1220-1225
28. Allen, T. M. (1994) Long-circulating (sterically stabilised) liposomes for targeted drug delivery. *TIPS* **15**, 215-220
29. Nabel, E. G., Plautz, G., and Nabel, G. J. (1990) Site-specific gene expression in vivo by direct gene transfer into the arterial wall. *Science* **249**, 1285-1288
30. Mizuno, M., Yoshida, J., Sugita, K., Inoue, I., Seo, H., Hayashi, Y., Koshizaka, T., and Yagi, K. (1990) Growth inhibition of glioma cells transfected with the human β-interferon gene by liposomes coupled with a monoclonal antibody. *Cancer Res.* **50**, 7826-7829
31. Stavridis, J. C., Deliconstantinos, G., Psallidopoulos, M. C., Armenakas, N. A., Hadjiminas, D. J., and Hadliminas, J. (1986) Construction of transferrin-coated liposomes for in vivo transport of exogenous DNA to bone marrow erythroblasts in rabbits. *Exp. Cell Res.* **164**, 568-572
32. Walther, F. J., David-Cu, R., Supnet, M. C., Longo, M. L., Fan, B. R., and Bruni, R. (1993) Uptake of antioxidants in surfactant liposomes is enhanced by SP-A. *Am. J. Physiol.* **265**, L330-L339
33. Morishita, R., Gibbons, G. H., Kaneda, Y., Ogihara, T., and Dzau, V. J. (1993) Novel in vitro gene transfer method for study of local modulators in vascular smooth muscle cells. *Hypertension* **21**, 894-899
34. Bagai, S., and Sarkar, D. P. (1993) Targeted delivery of hygromycin B using reconstituted Sendai viral envelopes lacking haemagglutinin-neuraminidase. *FEBS Lett.* **326**, 183-188
35. Schreier, H., Gonzalez-Rothi, R. J., and Stecenko, A. A. (1993) Pulmonary delivery of liposomes. *J. Contr. Release* **24**, 209-223
36. Zhu, N., Liggitt, D., Liu, Y., and Debs, R. (1993) Systemic gene expression after intravenous DNA delivery into adult mice. *Science* **261**, 209-211
37. Michael, S. I., and Curiel, D. T. (1994) Strategies to achieve targeted gene delivery via the receptor-mediated endocytosis pathway. *Gene Ther.* **1**, 223-232
38. Ferkol, T., Lindberg, G. L., Chen, J., Perales, J. C., Crawford, D. R., Ratnoff, O. D., and Hanson, R. W. (1993) Regulation of the phosphoenolpyruvate carboxykinase/human factor IX gene introduced into the livers of adult rats by receptor-mediated gene transfer. *FASEB J.* **7**, 1081-1091
39. Rosenkranz, A. A., Yachmenev, S. V., Jans, D. A., Serebryakova, N. V., Murav'ev, V. I., Peters, R., and Sobolev, A. S. (1992) Receptor-mediated endocytosis and nuclear transport of a transfecting DNA construct. *Exp. Cell Res.* **199**, 323-329
40. Chen, J., Gamou, S., Takayanagi, A., and Shimizu, N. (1994) A novel gene delivery system using EGF receptor-mediated endocytosis. *FEBS Lett.* **338**, 167-169
41. Batra, R. K., Wang-Johanning, F., Wagner, E., Garver, R. I., and Curiel, D. T. (1994) Receptor-mediated gene delivery employing lectin-binding specificity. *Gene Ther.* **1**, 255-260
42. Michael, S. I., Huang, C.-H., Romer, M. U., Wagner, E., Hu, P.-C., and Curiel, D. T. (1993) Binding-incompetent adenovirus facilitates molecular conjugate-mediated gene transfer by the receptor-mediated endocytosis pathway. *J. Biol. Chem.* **268**, 6866-6869
43. Lozier, J. N., Thompson, A. R., Hu, P.-C., Read, M., and Brinkhouse, K. M. (1994) Efficient transfection of primary cells in a canine hemophilia B model using adenovirus-polylysine-DNA conjugates. *Hum. Gene Ther.* **5**, 313-322
44. Wagner, E., Zenke, M., Cotten, M., Beug, H., and Birnstiel, M. L. (1990) Transferrin-polycation conjugates as carriers for DNA uptake into cells. *Proc. Natl. Acad. Sci. USA* **87**, 3410-3414
45. Yang, Y., Nunes, F. A., Berencsi, K., Furth, E., Gonczol, E., and Wilson, J. M. (1994) Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proc. Natl. Acad. Sci. USA* **91**, 4407-4411
46. Hoeben, R. C., Migchielson, A. A. J., van der Jagt, R. C. M., van Ormondt, H., and van der Eb, A. J. (1991) Inactivation of the Moloney murine leukemia virus long terminal repeat in murine fibroblast cell lines is associated with methylation and is dependent on its chromosomal position. *J. Virol.* **65**, 904-912
47. Cox, G. A., Cole, N. M., Matsumura, K., Phelps, S. F., Hauschka, S. D., Campbell, K. P., Faulkner, J. A., and Chamberlain, J. S. (1993) Overexpression of dystrophin in transgenic mdx mice eliminates dystrophic symptoms without toxicity. *Nature* **364**, 725-729
48. Maxwell, I. H., Glode, L. M., and Maxwell, F. (1991) Expression of the diphtheria toxin A-chain coding sequence under the control of promoters and enhancers from immunoglobulin genes as a means of directing toxicity to B-lymphoid cells. *Cancer Res.* **51**, 4299-4304
49. Jahroudi, N., and Lynch, D. C. (1994) Endothelial-cell-specific regulation of von Willebrand factor gene expression. *Mol. Cell. Biol.* **14**, 999-1008
50. Hatzoglou, M., Lamers, W., Bosch, F., Wynshaw-Boris, A., Clapp, D. W., and Hanson, R. W. (1990) Hepatic gene transfer in animals using retroviruses containing the promoter from the gene for phosphoenolpyruvate carboxykinase. *J. Biol. Chem.* **265**, 17285-17293
51. Vile, R. G., Miller, N., Chernajovsky, U., and Hart, I. R. (1994) A comparison of the properties of different retroviral vectors containing the murine tyrosinase promoter to achieve transcriptionally targeted expression of the HSVtk or IL-2 genes. *Gene Ther.* **1**, 1-10
52. Friedman, J. M., Babiss, L. E., Clayton, D. F., and Darnell, J. E. (1986) Cellular promoters incorporated into the adenovirus genome: cell specificity of albumin and immunoglobulin expression. *Mol. Cell. Biol.* **6**, 3791-3797
53. Brady, H. J. M., Miles, C. G., Pennington, D. J., and Dzierzak, E. A. (1994) Specific ablation of human immunodeficiency virus Tat-expressing cells. *Proc. Natl. Acad. Sci. USA* **91**, 365-369
54. Culver, K. W., Ram, Z., Wallbridge, S., Ishii, H., Oldfield, E. H., and Blaese, R. M. (1992) In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumours. *Science* **256**, 1550-1552
55. Miyao, Y., Shimizu, K., Moriuchi, S., Yamada, M., Nakahira, K., Nakajima, K., Nakao, J., Kuriyama, S., Tsujii, T., Mikoshiba, K., Hayakawa, T., and Ikenaka, K. (1993) Selective expression of foreign genes in glioma cells: use of the mouse myelin basic gene promoter to direct toxic gene expression. *J. Neurosci. Res.* **36**, 472-479
56. Huber, B. E., Richards, C. A., and Krenitsky, T. A. (1991) Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: an innovative approach for cancer therapy. *Proc. Natl. Acad. Sci. USA* **88**, 8039-8043
57. Vile, R. G., and Hart, I. R. (1993) Use of tissue-specific expression of the herpes simplex virus thymidine kinase gene to inhibit growth of established murine melanomas following direct intra-tumoral injection of DNA. *Cancer Res.* **53**, 3860-3864
58. Harris, J. D., Gutierrez, A. A., Hurst, H. C., Sikora, K., and Lemoine, N. R. (1994) Gene therapy for cancer using tumour-specific prodrug activation. *Gene Ther.* **1**, 170-175
59. Mayor, H. D. (1993) Defective parvoviruses may be good for your health. *Prog. Med. Virol.* **40**, 193-205
60. Guetta, E., Graziani, Y., and Tal, J. (1986) Suppression of Ehrlich ascites tumors in mice by minute virus of mice. *J. Natl. Cancer Inst.* **76**, 1177-1180
61. Dupont, F., Tenenbaum, L., Guo, L.-P., Spegelaere, P., Zeicher, M., and Rommelaere, J. (1994) Use of an autonomous parvovirus vector for selective transfer of a foreign gene into transformed human cells of different origin and its expression therein. *J. Virol.* **68**, 1397-1406
62. Huxley, C. (1994) Mammalian artificial chromosomes: a new tool for gene therapy. *Gene Ther.* **1**, 7-12
63. Arbones, M. L., Austin, H. A., Capon, D. J., and Greenburg, G. (1994) Gene targeting in normal somatic cells: inactivation of the interferon-γ receptor in myoblasts. *Nature Genet.* **6**, 90-97
64. Shelling, A. N., and Smith, M. G. (1994) Targeted integration of transfected and infected adeno-associated virus vectors containing the neomycin resistance gene. *Gene Ther.* **1**, 165-169
65. Samulski, R. J. (1993) Adeno-associated virus: integration at a specific chromosomal locus. *Curr. Opin. Genet. Dev.* **3**, 74-80
66. Chiorini, J. A., Weitzman, M. D., Owens, R. A., Urcelay, E., Safer, B., and Kotin, R. M. (1994) Biologically active Rep proteins of adeno-associated virus type 2 produced as fusion proteins in *Escherichia coli*. *J. Virol.* **68**, 797-804
67. Flotte, T. R., Afione, S. A., Conrad, C., McGrath, S. A., Solow, R., Oka, H., Zeitlin, P. L., Guggino, W. B., and Carter, B. J. (1993) Stable in vivo expression of the cystic fibrosis transmembrane conductance regulator with an adeno-associated virus vector. *Proc. Natl. Acad. Sci. USA* **90**, 10613-10617
68. McDonald, J. F. (1993) Evolution and consequences of transposable elements. *Curr. Opin. Genet. Dev.* **3**, 855-864
69. Burns, N. R., Saibil, H. R., White, N. S., Pardon, J. F., Timmins, P. A., Richardson, S. M., Richards, B. M., Adams, S. E., Kingsman, S. M., and Kingsman, A. J. (1992) Symmetry, flexibility and permeability in the structure of yeast retrotransposon virus-like particles. *EMBO J.* **11**, 1155-1164
70. Chakraborty, A. K., Zink, M. A., Boman, B. M., and Hodgson, C. P. (1993) Synthetic retrotransposon vectors for gene therapy. *FASEB J.* **7**, 971-977
71. Voytas, D. F., and Boeke, J. D. (1993) Yeast retrotransposons and tRNAs. *Tiends Genet.* **9**, 421-427

THE JOURNAL OF INFECTIOUS DISEASES • VOL. 153, NO. 4 • APRIL 1986
 0022-1899/86/5304-0004$01.00

Relation of *Campylobacter pyloridis* to Gastritis and Peptic Ulcer

George E. Buck, William K. Gourley, Won K. Lee, Kalyanam Subramanyam, Joan M. Latimer, and Anthony R. DiNuzzo

From the Departments of Pathology and Internal Medicine, The University of Texas Medical Branch at Galveston, Galveston, Texas

Biopsy specimens from the gastric antral mucosa of 50 patients with upper gastrointestinal complaints were studied by light and electron microscopy and culture. Of 46 assessable specimens, seven were histologically normal, and 39 showed evidence of gastritis. Twenty-seven of the specimens with evidence of gastritis (69%) contained spiral bacteria, whereas only one of the normal specimens (14%) contained these bacteria ($P = .02$). Of 17 patients found to have gastric ulcers, 10 (59% [$P > .10$]) also had spiral bacteria. The bacteria could be seen scattered over the surface of the epithelial cells and just under the layer of mucus but were rarely found inside the epithelial cells. Curved or spiral gram-negative bacilli were isolated from 10 of the specimens on chocolate agar incubated at 37 C under microaerophilic conditions. The bacteria resembled the organism recently named *Campylobacter pyloridis* by other investigators.

Gastrititis and peptic ulcers are major conditions that affect numerous individuals every year. The etiology and pathogenesis of these conditions are not fully understood at present. The term *gastritis* includes a group of inflammatory states of the stomach differentiated according to the area affected, extent of damage, and type of inflammatory cell present [1]. The etiology is known in some of these conditions, but in others it is less well defined. The physiological basis of peptic ulcers is not entirely clear, but it is thought to be related to the relative balance of acid production to the resistance of mucous lining covering the gastric epithelium. Stress and other unknown factors are thought to affect this relative balance [2].

Recently, Marshall and Warren [3] and Warren and Marshall [4] presented evidence that a new species of *Campylobacter*, named *Campylobacter pyloridis* [5], was associated with gastritis and peptic ulcers; it was suggested that this organism might participate in causing these diseases. Other preliminary reports have also appeared with similar results [6–9]. The work reported here was initiated to confirm this observation and to explore further the characteristics of this organism.

Received for publication 1 July 1985, and in revised form 24 October 1985.

Informed consent was obtained from all patients participating in this study.

Please address requests for reprints to Dr. George E. Buck, Microbiology Division, Clinical Laboratories (G43), Department of Pathology, The University of Texas Medical Branch at Galveston, Galveston, Texas 77550.

Materials and Methods

Patients studied. A total of 50 consecutive patients presenting to the Gastroenterology Clinic at the University of Texas Medical Branch (Galveston, Tex) was studied. The age range of the patients was 22–77 years, and the median age was 50 years. There were 25 men and 25 women. The endoscopy procedure was performed to investigate abdominal symptoms such as epigastric pain, nausea, vomiting, rectal bleeding, etc. Five of the patients were receiving antibiotics at the time of endoscopy: two trimethoprim/sulfamethoxazole, one erythromycin, one cephradine, and one ampicillin. Any individual with coagulopathy or requiring emergency endoscopy was automatically excluded from the study. With each patient the gastric antrum was visualized by endoscopy, and in addition to a routine biopsy specimen taken for routine diagnosis, several additional pieces of tissue, ~1 mm in diameter, were excised.

Light microscopy. After the specimens were obtained, one piece was fixed in 10% buffered formalin for light microscopy, another was saved for electron microscopy by fixation with 2% glutaraldehyde in 0.2 *M* cacodylate buffer for 2 hr and then stored in the same buffer at 4 C until processed, and a third piece was cultured. The formalin-fixed specimens were embedded in paraffin with use of standard procedures, sectioned, and stained with hematoxylin and eosin, gram stain, and Dieterli's silver stain. The slides were coded with a random number and examined in a blind manner to prevent bias. Each slide was examined independently by two patholo-

gists (W.K.L. and W.K.G.), who then reconciled any differences before the results were correlated. The diagnosis of gastritis was based on the amount and character of inflammatory infiltrate in the lamina propria or gastric glands. The absence or presence of intestinal metaplasia was noted. Categorization was not difficult when acute inflammation of the tissue was present. When such inflammation was absent, assessment of gastritis was more subjective and difficult to evaluate consistently. Therefore the cases were classified into three categories: normal, clear-cut gastritis, and borderline chronic gastritis. The last term was used when the only abnormality was an increased number of lymphocytes and plasma cells in the lamina propria, when compared with specimens classified as normal.

Electron microscopy. The specimens were post-fixed in 1% osmic acid, washed, dehydrated in a graded series of ethanol, and embedded in epoxy plastic. Plastic-embedded sections 1-μm thick were stained with methylene blue–azure II and basic fuchsin [10].

Isolation and characterization of bacteria. Biopsy specimens were inoculated onto chocolate agar plates made with GC medium base supplemented with 0.025% yeast extract, 1% hemoglobin, and 1% V-enrichment (Austin Biological Laboratories, Austin, Tex). The plates were incubated in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2 at 37 C and examined daily for seven days. The atmosphere was generated by placing the plates in a torbal jar (American Scientific, Houston), evacuating the jar with the laboratory vaccuum line, and refilling the jar with a commercially prepared gas mixture (Linde Division, Union Carbide, Somerset, NJ). A square of absorbent paper containing several drops of tap water was included in the jar to supply additional moisture.

Biochemical characteristics of the isolates were determined as follows. Oxidase and catalase tests were performed in the standard manner [11]. Glucose fermentation was determined with purple broth base containing 1% glucose. Production of H_2S was determined with use of triple sugar iron agar. Indole production was determined by incubation of the isolates in tryptone broth, extraction of the broth with xylene, and addition of Erlich's reagent [11]. Reduction of nitrate was determined with use of nitrate broth. Hippurate hydrolysis was tested by the method described by Lior [12]. Susceptibility to nalidixic acid and cephalothin were tested with use of 30-μg disks (General Diagnostics, Santurce, Puerto Rico) on Columbia blood agar base. Where necessary, the media were supplemented with horse serum at a final concentration of 5%, except in the case of agar slants, where a few drops were added to the slant.

Growth at 25, 37, and 42 C was assessed by inoculation of the isolates onto chocolate agar plates and incubation of the plates in a commercially supplied plastic bag with a gas-generating vial (Bio-Bag environmental chamber, type cfj; Marion Laboratories, Kansas City, Mo) at these temperatures. To determine whether the isolates grew anaerobically, we streaked them onto chocolate agar and incubated them at 37 C in a similar plastic bag that generates an anaerobic atmosphere (Bio-Bag, type A, Marion Laboratories). A similar plate was also incubated in ambient air at 37 C. Additional growth characteristics of several isolates were examined by inoculating them to various media that were plain (without supplementation) or with blood or serum supplements. Three to seven isolates were streaked onto the plates, and the plates were incubated in a microaerophilic atmosphere at 37 C for three days.

Isolates were tested for antibiotic susceptibility by agar dilution [13] on Mueller-Hinton agar containing 10% horse serum and incubation in a microaerophilic atmosphere at 37 C. Two concentrations of each antimicrobial agent, corresponding to the breakpoint concentrations, were tested. The following antimicrobial agents and concentrations were tested: vancomycin, 5 and 10 μg/ml; erythromycin, 4 and 8 μg/ml; chloramphenicol, 12.5 and 25 μg/ml; clindamycin, 1 and 2 μg/ml; cephalothin, 8 and 16 μg/ml; gentamicin, 4 and 8 μg/ml; and tetracycline, 6 and 12 μg/ml.

Statistical methods. Statistical analyses were

Table 1. Correlation between inflammation in antral biopsy specimens and the presence of spiral bacteria.

Histological category	No. of patients			
		Spiral bacteria		
	Total	Positive	Negative	p^*
Normal	7	1	6	. . .
Borderline gastritis	11	9	2	.02
Gastritis	20	15	5	.02
Gastritis with intestinal metaplasia	8	3	5	.67
Total with gastritis	39	27	12	.02
Total	46	28	18	. . .

* In comparison with normal tissue.

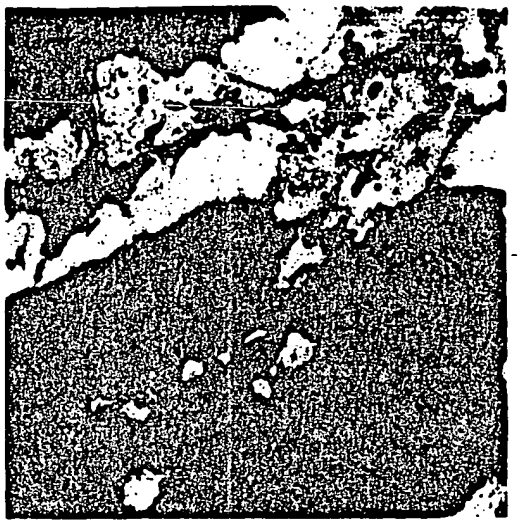


Figure 1. Histological section of a biopsy specimen from the gastric antrum stained with Dieterli's silver stain (×950).

done with the χ^2 test with Yates's correction for continuity.

Results

Of the 50 patients evaluated, 4 were excluded because their biopsy specimens were too small for adequate evaluation, 7 were found by histological examination to have normal gastric mucosa, and the remaining 39 patients had some degree of gastritis. Spiral bacilli were found in 27 (69%) of these 39 patients and in only one (14%) of the seven patients with histologically normal gastric mucosa ($P = .02$). The presence of bacilli and category of inflammation are compared in table 1. A total of 17 (37%) of the 46

Figure 2. Gram-stained smear from a biopsy specimen shows numerous spiral or curved bacteria (×950).

Figure 3. Electron micrograph of the luminal surface of a gastric foveolar crypt. Transversely and longitudinally sectioned spiral bacilli (arrows) are adjacent to the surface of mucous epithelial cells. Bar = 1 μm.

Table 2. Growth characteristics of strains of *Campylobacter* isolated from human gastric tissue.

Isolate no.	Growth in			Growth at		
	Ambient air	Anaerobic atmosphere	5% CO_2	25 C	37 C	42 C
2	−	±	+	−	+	+
5	−	−	+	−	+	+
6	−	−	+	−	+	+
7	−	±	+	−	+	+
10	−	±	±	−	+	+
11	−	−	±	−	+	+
16	−	+	+	−	+	+
17	−	−	+	−	+	+
18	−	+	+	−	+	+
19	−	+	+	−	+	−

patients had a gastric ulcer or erosion visible on endoscopic examination, and spiral bacilli were found in 10 (59%). There was no significant difference in the frequency of bacilli in patients with ulcer or erosion compared with patients with histological gastritis ($P > .10$). There was also no significant difference between patients with ulcer and those with histologically normal mucosa. Of the five patients who were receiving antibiotics at the time of the study, the organism was either isolated or seen in tissue from three, and tissue from the other two was negative. None of the patients with normal histology was receiving antibiotics.

The bacteria could be seen most easily in tissue sections with silver stain (figure 1), but they could also be seen with Brown-Brenn tissue gram stain or even with the routine hematoxylin and eosin stain. They occurred on the surface of the epithelial cells and in the lumen, under the mucous layer. In many cases the bacteria could be readily demonstrated in a gram-stained smear directly from the tissue (figure 2). Examination of the tissues by electron microscopy (figure 3) confirmed that the bacteria occurred primarily in close association with the surface of the epithelial cells.

Curved, gram-negative bacilli were isolated from 19 of the specimens. The isolates grew slowly, requiring four to seven days for recovery in culture, but on subculture the colonies reached maximal size in about three days. The colonies were circular, convex, translucent, and nonpigmented, and the maximal size was ~0.5 mm. All isolates were oxidase and catalase positive. Staining of flagella by the Ryu method [14] showed that the cells had a tuft of polar flagella. Ten of the isolates were characterized further. Growth in different atmospheric conditions or at 25, 37, or 42 C is shown in table 2. None of the strains fermented glucose, hydrolyzed hippurate, or produced indole or H_2S. Two of the strains reduced nitrate, but the others were negative. All were resistant to nalidixic acid and susceptible to cephalothin by the disk diffusion test. These characteristics fit those previously published for *C. pyloridis* [3–5]. A unique feature of these organisms was that the morphology in pure culture differed slightly from that seen in tissue. In pure culture (figure 4) the bacteria were frequently more rodlike, and bizarre shapes, such as U-shaped cells or circular cells, could be seen. Eight of the isolates were further examined for susceptibility to common antimicrobial agents with use of agar dilution methodology and standard breakpoints for interpretation [12]. All of these isolates were resistant to vancomycin (MIC, ≤1 μg/ml) but susceptible to erythromycin (MIC, ≤4 μg/ml), clindamycin (MIC, ≤1 μg/ml), cephalothin (MIC, ≤8 μg/ml), gentamicin (MIC, ≤4 μg/ml), and tetracycline (MIC, ≤6 mg/ml).

An experiment (table 3) was performed to investigate the growth on various media and the requirement for blood or serum. Three to seven isolates were streaked on the various media, and growth was assessed after three days at 37 C under microaerophilic

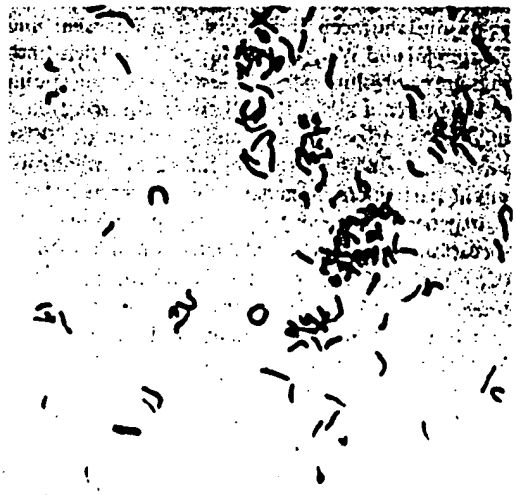


Figure 4. Gram-stained preparation from a colony of bacteria isolated from a gastric biopsy specimen. The cells are more loosely curved and exhibit unusual morphological forms (×950).

Table 3. Growth of representative isolates on various media.

Medium	No supplement	5% serum		5% blood	
		Horse	Sheep	Rabbit	Sheep
Chocolate	+	ND	ND	ND	ND
Thayer-Martin	+	ND	ND	ND	ND
GC agar	+	+	+	+	+
Columbia	0	+	+	+	+
Trypticase soy	0	+	+	+	+
Brain-heart infusion	0	+	+	+	+
Brucella	0	+	+	+	+
Nutrient agar	0	0	0	0	0

NOTE. Growth was rated on the following scale: + = growth; 0 = no growth. ND = not done.

conditions. All of the isolates tested grew on GC agar base without any supplement but not on the other media tested unless blood or serum was present. No growth was obtained with use of nutrient agar, even with blood or serum supplementation.

Discussion

Spiral bacteria were originally observed in human gastric tissue many years ago [15], but early investigations to confirm this observation and to examine its significance yielded conflicting results [16, 17]. Therefore these observations were ignored for many years until the recent investigations of Marshall and Warren [3] and Warren and Marshall [4]. Their findings are of revolutionary interest because they point to a significant association between the occurrence of gastritis and peptic ulcers and the presence of these bacteria, a finding implying that these organisms might participate in causing these diseases.

In general, our results are consistent with their observations. We found spiral bacteria in only 1 (14%) of 7 patients with histologically normal gastric mucosa but in 27 (69%) of 39 patients with gastritis and 10 (59%) of 17 of patients with gastric ulcer. Statistical analysis showed a significant difference between the normal group and patients with gastritis but not between the normal group and patients with ulcer. Therefore our results indicate a distinct association of the bacteria with gastritis; this conclusion is in agreement with other recent findings [5–9]. Our results failed to show a definite association with ulcers. This conclusion agrees with the results of Rollason et al. [6] and Burnett et al. [9] but conflicts with the results of other investigations [5, 7, 8]. As larger numbers of patients are studied, these conflicts may well resolve.

Because the patients examined were consecutive and essentially unselected, some of them were receiving antibiotics at the time of examination. Antibiotic use apparently had minimal effect on the results because three of these patients were positive for bacteria, and two were negative. None of these patients was in the group with normal histological findings.

The fact that these organisms can be found in electron micrographs in large numbers and in close association with the surface of epithelial cells deep in foveolar crypts suggests they are not merely mouth flora that contaminated the biopsy specimens on withdrawal of the endoscope. However, although these bacteria are clearly associated with gastritis and probably also associated with gastric ulcers, their significance as far as causing these diseases is still in question. In this regard we have preliminary information (authors' unpublished observation) that inflammatory cells are present in the gastric lumen ingesting these baceria, thus suggesting an active effort on the part of the host to remove these bacteria.

The organism recovered from the tissues has unique characteristics. It is oxidase and catalase positive and grows either under microaerophilic conditions or in 5% CO_2 but not in ambient air, and most isolates do not grow under strict anaerobic conditions. A unique characteristic is its change in morphology when grown in vitro. Because of this alteration we initially wondered whether this organism really was the same as that seen in tissues. However, the isolates were found in high numbers in many of the specimens and were not found in routine cultures of the mouth from 20 randomly selected individuals (authors' unpublished observation). Therefore we believe the organisms isolated are the same as those seen in tissue, although this conclusion cannot be proved at the moment. One means of substantiating this proposal would be to make antibody to the isolates and determine whether the antibody reacts with sprial bacilli found in tissue sections. Also, preliminary comparison of our isolates with several strains supplied by Dr. B. J. Marshall shows that they are similar in characteristics.

Although this organism has recently been named *C. pyloridis*, there are several characteristics that do not really fit the genus *Campylobacter*. First, the bacteria have multiple polar flagella rather than a single flagellum, and, in addition, according to Marshall and Warren [3], the flagella are sheathed.

Second, the unusual morphology in pure culture is different from any other species of *Campylobacter* known. Third, the failure to grow on most media without added serum is a feature also not found in the other *Campylobacter* spp. These characteristics are sufficiently different from known *Campylobacter* spp. that this organism may actually represent a new genus. Genetic characterization would settle this question.

References

1. MacDonald WC, Rubin CE. Cancer, benign tumors, gastritis, and other gastric diseases. In: Isselbacher KJ, Adams RD, Braunwald E, Petersdorf RG, Wilson JD, ed. Harrison's principles of internal medicine. 9th ed. New York: McGraw-Hill, 1980:1385–92
2. McGuigan JE. Peptic ulcer. In: Isselbacher KJ, Adams RD, Braunwald E, Petersdorf RG, Wilson JD. ed. Harrison's principles of internal medicine. 9th ed. New York: McGraw-Hill, 1980:1371–84
3. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis, and peptic ulceration [letter]. Lancet 1984;1:1311–4
4. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis [letter]. Lancet 1983;1:1273–5
5. Marshall BJ, Royce H, Annear DI, Goodwin CS, Pearman JW, Warren JR, Armstrong JA. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. Microbios 1984;25:83–8
6. Rollason TP, Stone J, Rhodes JM. Spiral organisms in endoscopic biopsies of the human stomach. J Clin Pathol 1984;37:23–6
7. Langenberg ML, Tytgat GNJ, Schipper MEI, Rietra PJGM, Zanen HC. *Campylobacter*-like organisms in the stomach of patients and healthy individuals [letter]. Lancet 1984; 1:1348
8. McNulty CAM, Watson DM. Spiral bacteria of the gastric antrum [letter]. Lancet 1984;1:1068–9
9. Burnett RA, Forrest JAH, Girdwood RWA, Fricker CR. *Campylobacter*-like organisms in the stomach of patients and healthy individuals [letter]. Lancet 1984;1:1349
10. Mackay GR, Mead ML. A simple dichromatic strain for plastic embedded tissues. In: Arceneaux C, ed. Proceedings of the Electron Microscopy Society of America. 28th Annual Meeting. Baton Rouge, La: Claitor's Publishing, 1970:296–7
11. Paik G. Reagents, stains and miscellaneous test procedures. In: Lennette EH, Balows A, Hausler WJ Jr, Truant JP, eds. Manual of clinical microbiology. 3rd ed. Washington, DC: American Society for Microbiology, 1980:1000–24
12. Lior H. New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis*. J Clin Microbiol 1984;20:636–40
13. Washington JA II, Sutter VL. Dilution susceptibility test: agar and macro-broth dilution procedures. In: Lennette EH, Balows A, Hausler WJ Jr, Truant JP, eds. Manual of clinical microbiology. 3rd ed. Washington, DC: American Society for Microbiology, 1980:453–8
14. Kodaka H, Armfield AY, Lombard GL, Dowell VR. Practical procedure for demonstrating bacterial flagella. J Clin Microbiol 1982;16:948–52
15. Salomon H. Ueber das Spirillum des Saugetiermagens und sein Verhalten zu den Belegzellen. Zentralblatt für Bakteriologie 1896;19:433–42
16. Palmer ED. Investigation of the gastric mucosa spirochetes of the human. Gastroenterology 1954;27:218–20
17. Freedberg AS, Barron LE. The presence of spirochetes in human gastric mucosa. American Journal of Digestive Diseases 1940;7:443–5